

## What can the Party do for science?

*Next week's special conference of the Soviet Communist Party will probably have the wit to confirm Mr Mikhail Gorbachev as its leader. Will it have the courage to set Soviet science free?*

NEXT Tuesday, 28 June, is a red-letter day, for that is when the special conference of the Soviet Communist Party arranged last year will be convened in Moscow. Although there have been occasions since last November when it seemed as if the conference would critically determine whether Mr Mikhail Gorbachev, the party's general-secretary, would be allowed to push ahead with his advertised reforms, the past few weeks have been encouraging. During this run-up to the conference, *glasnost* has been carried so much further than seemed possible even a year ago as to suggest great confidence among the party's reformers. And while many of the plans for social and economic change recently made public await the endorsement of the conference, the fact that they have been made public in any shape or form is almost a proof that change will come about. It may also, of course, have helped Mr Gorbachev to win the endorsement (as they say in US politics) of President Ronald Reagan.

So far, there is little sign of how the pattern of Soviet science will be changed by the conference and by its decisions. Central though science and technology are held to be in the Marxist state, and influential though the scientific establishment is throughout the Soviet Union, there is little that a party conference can say, let alone do, to rid Soviet science of the encumbrances with which it is saddled. It is a problem in hysteresis. When the going has been rough, Soviet governments have dealt generously with the scientific establishment, repeatedly augmenting its stock of power and influence, in return for promissory notes to make that vast country fertile, or prosperous, or militarily strong (on which the scientific community seems to have delivered). At other times, the establishment has kept its power, but not much has been asked of it.

For generous governments have frequently acknowledged that some promises cannot reasonably be kept. Nobody, for example, could be held to account for failing to keep a promise to make the Soviet Union prosperous when the inefficiencies of the economic system are as flagrant as the world (and even the Soviet government) knows them to be. So the Soviet research establishment has been forced into an invidious position: it has more power than it can usefully exercise, but, given the superabundance of its power, it exercises it vicariously. It has also, by being seen to promise what it could not do, acquired a reputation for being ineffectual, which is not the best recipe for commanding the respect of ordinary mortals, its own underlings (working scientists) in particular. Neither the establishment nor successive Soviet governments seems to have calculated that what the research community in the Soviet Union most needs is a greater modicum of freedom than at present to tackle problems that seem interesting, and some (not necessarily all) of the resources that would be necessary for success.

The resignation, earlier this month, of no fewer than fifteen members of the Praesidium of the Soviet Academy is in this connection probably symbolic (see page 692). Old men must indeed make way for younger men (or even women, Soviet readers might usefully note), but it will have helped the academy to have demonstrated this truth on the eve of probably the most important occasion in the political life of the Soviet Union in fifty years. But the crying need, for the past half century and

now, is for a means by which ordinary scientists at the bench can be more productive. With *glasnost*, the sense of being free may be arranged with relative ease. It will be more difficult to arrange that people have the tools of the trade to enable them to be productive. But, even more important, they need to know that the hardships they share with their fellows in the Soviet Union are not exclusively their responsibility. If there is a shortage of water-melons, or of toothpaste, and if it is true that science might be organized so as in due course to remove it, might it nevertheless not be more productive that the water-melons and toothpaste should be left for others to worry about, and that the creative parts of the Soviet scientific enterprise should tackle interesting problems? □

## Blinkered UK students

*The British school examinations system is about to be modified, but not reformed.*

NOT before time, the British educational system, under external pressure on all fronts, has finally begun to worry about a substantial, but internal, issue to which it should have paid much more attention in years past: the question of how young people in Britain are prepared for entry into higher education (or, for that matter, into adulthood). Until this decade, the most glaring defect of the British educational system has also been the proudest boast of many who teach in schools as well as the crutch with the help of which teachers in higher education have reckoned that three years are usually sufficient to turn undergraduates into graduates.

The scandal is, of course, the system by which young people are virtually compelled to follow specialized curricula while still at secondary school, commonly from the age of thirteen (but some don intellectual blinkers even earlier). The point has now been reached at which it is agreed that this iniquitous system should be reformed, but unsurprisingly there are disagreements about the manner in which change should be brought about — and none of the reforms being canvassed is sufficiently radical to endure for long.

The issue is arcane, and peculiar to Britain. Half a century ago, when the British participation rate in higher education was between 3 and 5 per cent and when much of higher education was carried out in vocational colleges of various kinds (preparation for teaching in schools, or for a career as a technician), it became convenient to supplement a public examination at sixteen with a school-leaving examination enabling students to demonstrate aptitudes in particular fields, usually at eighteen. Until the 1930s, individual universities would decide between prospective students on the basis of the first of these examinations and, usually, an examination organized by themselves (with which Oxbridge persists). But then tidiness suggested that the second examination, originally (and, in Scotland still) the "higher" certificate, now renamed the "advanced" or "A-level" certificate, should become the basis for telling who should go to university. The consequence has been that academically ambitious students have been forced into a mould in which their



last two years of secondary schooling have been a kind of rehearsal of undergraduate studies. Until quite recently, it has been the custom for university departments to make success in specified A-level subjects a necessary (but not necessarily a sufficient) condition of entry.

The consequences of this system have done as much to undermine the quality of British education as any shortage of funds externally imposed, but are only now being generally recognized. Almost all young people have been educated narrowly, however closely their schools have been able to simulate higher education. By being required to "choose" their "subjects" before they can form a judgement of the kinds of skills they will need to acquire, most young people in Britain have also been turned into suspected misfits; having settled for "science" or something else, they are constantly looking over their intellectual shoulders in ignorance of what they never studied, wondering whether their choice was wise. The damage done to those who are blinkered in this way, but who then never go on to higher education, is almost unthinkable. But there is also ample evidence that the system has robbed the scientific professions of able people; given the endemic relative shortage of gifted science teachers in British schools, it is inevitable that other disciplines should have won an unduly high proportion of susceptible young hearts and minds.

Only in the past few years has it been generally recognised in Britain that this system must change. One of the few virtues of the government's Education Reform Bill is that there will in future be a national curriculum (up to the age of sixteen) legislating for some kind of balance in the education of the young. The Secretary of State for Education and Science, Mr Kenneth Baker, is also firmly backing a new system of school-leaving examinations in which intending entrants to higher education will ordinarily follow five (rather than three) courses, not all of them of equal weight or in the same direction.

Not everybody is happy. One of Baker's advisory committees, charged with defining the ideal pattern and preferring six courses of equal weight, has been rebuffed on the good grounds that the new system will be a going concern in the next academic year. What tends to be forgotten, in this confused tale, is that the system about to be introduced (and for which students are already following new courses) is essentially that rejected hotly by British universities just over twenty years ago. It is forgivable that Baker may seem to have lost patience with some of his constituents.

That does not imply that the new regime will be markedly better than the old. Indeed, in many ways it will perpetuate some of the worst features of British academic life, among which one of the most corrosive is the snobbery of those whose academic pretensions are required. The new examinations scheme also takes far too little account of how circumstances have changed during the two decades since it was originally (but abortively) designed. Despite recent cuts, the participation rate in higher education has now grown to 15 per cent, while many of the institutions providing this service find themselves threatened with externally enforced change.

Some institutions still boasting of their research are clearly destined to become largely teaching institutions. What reason can there be to hope that the same system of school-leaving examinations can serve as a basis (by defining necessary but insufficient conditions) for entry to the variety of higher education institutions now belatedly emerging in Britain? And will there not, in the new system, be even more substance in the old academic complaint that students reaching university less well drilled in what they will be studying cannot gain a first degree in just three years?

The simple way of dealing with this and other conundrums is to put back the clock to the time when the 16-plus examination, which will in future be a certificate of general education, was the necessary condition for entry into higher education, leaving it to universities and polytechnics separately to develop other criteria

for selecting those whom they would teach. Some universities would no doubt wish to follow the University of Keele in legislating for a four-year degree course for everybody, in which case the government should encourage them in that direction. Interestingly, others might wish to experiment with captive secondary schools as complements to the public education service. Sadly, while the British government seems willing to try out all kinds of schemes for giving schools greater autonomy, its face is set as hard as ever against such a measure of autonomy for higher education institutions, without which the benefits of the diversity now emerging will not materialize. □

## Pity poor Texaco?

*Proverbs such as "It never rains, but it pours!" apply to the world's unluckiest oil company.*

ONLY a few months ago, the US oil company Texaco was in the doghouse of the international oil business for having sought the protection of the US bankruptcy laws from a court judgement requiring a penalty of more than \$11,000 million (including interest). The origins of the dispute, going back to the early 1970s, are now irrelevant, but turned on a dispute between Texaco and a much smaller oil company called Penzoil over the means by which Texaco acquired valuable oil leases from a third company, Getty Oil.

During the protracted civil suit fought between Texaco and Penzoil, it seems not to have been disputed that Getty had first agreed to sell the disputed leases to Penzoil, but that Texaco eventually bought them for a higher price; the issue was whether Texaco had secured its purchase unfairly. The Texas courts eventually decided in favour of Penzoil, awarding compensation for the present value of past income stretching back over a decade and a half. To have paid up would have been to bankrupt: why not instead volunteer for bankruptcy instead, securing a moratorium from creditors which looking for a settlement. By the end of last year, having persuaded Penzoil that it is no easier to get water out of crushed stone than the integral variety, Texaco was let off with damages roughly a fifth of those originally awarded (by ordinary standards, far from chicken-feed) and seemed ready to face an unencumbered if leaner future.

It could hardly have been more wrong. From the outset, Texaco has featured prominently on the juicier pages of the US financial press. An immediate difficulty was the discovery that Mr Carl Icahn, most often described as a "corporate raider" but who happens also to have become chairman of Transworld Airlines after a stock-market tussle, had acquired 15 per cent of the company's shares. Another was that Mr Icahn proposed to buy the remaining shares at \$60 each, rather more than their present price of \$51 each. Last Friday, at a meeting of the company in Texas, votes were cast for and against Mr Icahn's proposal that four of his nominees should be elected to the company board. Meanwhile, Texaco seeks to make itself less vulnerable by selling assets — its Canadian subsidiary is on the block, while a distribution network in the United States is likely to be partly sold to Saudi Arabia.

Opinions differ on whether there is a moral in all this, unless it is the banal observation that companies are no less vulnerable than private persons if they find themselves at the wrong end of a court judgement. But if the judgement against Texaco was correctly argued, this train of events extends to companies the kind of good-neighbour view of life that people expect of other people. This, it will be recalled, is what happened to the Manville asbestos company and to the manufacturers of the "Dalkon shield" — an intrauterine contraceptive device, each of which faced overburdening product liability damages. At least in the United States, the courts expect of companies not only personal rectitude but remarkable farsightedness in anticipating dangers that might arise from their work. Do companies yet appreciate their difficulty? □



# US-Japan science accord is signed at last

- Exchange of scientists and information
- Patenting and classification fears denied

## Tokyo

COOPERATIVE relations in science and technology between the United States and Japan entered a new era on Monday, when Prime Minister Noboru Takeshita and US President Ronald Reagan signed the new US-Japan Science and Technology Agreement at the economic summit meeting in Toronto. The 28-page agreement is a detailed framework for future cooperation. It outlines priority areas for research including biotechnology, superconductors and automation, measures to foster the exchange of scientists and information and rules to protect intellectual property rights.

To increase exchanges of scientists, the two governments will "provide substantial numbers of competitive fellowships in science and engineering for foreign nationals at their respective centres of excellence" and "continue to improve foreign language training programs" for them.

Japan has been criticized by some US officials because far more Japanese scientists and engineers visit the United States than vice versa. Although funds to support several hundred fellowships for foreign scientists are now available, it remains to be seen whether the posts are filled — Japan has had difficulty attracting applicants for fellowship programmes with Europe and the United States.

Another problem is that about 80 per cent of Japan's research and development is carried out in the private sector, which tends to be closed to outsiders. Japan's Ministry of International Trade and Industry (MITI), which supports many major research programmes in collaboration with industry, may in future encourage the private sector to include foreigners in them. But until US scientists begin banging on Japan's doors, the imbalance seems destined to remain.

The rules regarding intellectual property rights have been contentious, often delaying the negotiations. The agreement is meant to cover several eventualities. If an invention is made purely as a result of exchange of information (as at a seminar), the inventor may obtain all rights in all countries. In the case of exchange of scientists, the country that accepts the visiting scientist(s) may obtain all rights in all countries to inventions arising provided that the host country is "expected to make a major and substantial contribution to the program of cooperative activity".

But when that proviso does not apply, the host country can obtain rights in its own and third countries, while the visiting inventor can obtain patent rights only in his own country.

As an illustration, young scientists requiring training in the host country would forfeit patent rights to the host — which applies to many Japanese scientists now visiting the United States. But, for joint research projects, intellectual property rights will be decided case-by-case, depending on how the costs are shared.

A high-level committee co-chaired by the Japanese Foreign Minister (or his designate) and the Science Adviser to the US President will review the operation of the agreement once a year, also assessing policies for the promotion of science and technology in each country and defining further initiatives.

In addition a working-level committee, chaired by the Japanese Foreign Ministry and the US State Department, with representatives from other agencies, will make recommendations to the high-level committee, issue an annual report and establish task forces to monitor the exchange of scientists and engineers and to improve access to information resulting from cooperation.

Finally, an advisory panel of eminent academic and industrial leaders from both countries will monitor the agreement and recommend areas for future research.

Concerns that the new agreement will clamp controls on research for reasons of national security seem unjustified. The agreement states that "no information or equipment classified for reasons of national defense will be utilized in cooperative activities" and both countries will support the "widest possible dissemination of information and equipment".

But the agreement does include a clause to prevent export of militarily-useful information and equipment to "unauthorized destinations". And critics say the agreement should not be viewed in isolation. The United States and Japan recently agreed that classified US patents could be taken out in Japan (*Nature*, 332, 669; 1988). There are fears this agreement may lead to restrictions of jointly developed technology. But a Japanese foreign ministry official said that the secret patent agreement is totally unrelated to the new agreement, and cannot be used to classify technology developed in Japan.

David Swinbanks

# Canada's high hopes

## Toronto

THE Canadian government is about to begin the long process that it hopes will eventually lead to a global "Law of the Atmosphere". Assembling in Toronto next week for the World Conference on the Changing Atmosphere: Implications for Global Security will be 300 scientists, economists, legal experts and policymakers from more than 40 countries.

Canada's backing for the conference stems not so much from an enthusiasm for the scientific issues (Canada's scientific scene is surveyed in this week's issue of *Nature*, pages 717-736) but from its geographical position. Stuck between the world's largest industrial nations, it receives pollution from both: in the south, acid rain comes courtesy of the United States and in the north, arctic haze arrives from the Soviet Union and northern Europe (see page 736). These problems have brought an unusually high environmental consciousness to Canada's politicians. Last year's success in negotiating the Montreal protocol restricting the output of chlorofluorocarbons encouraged politicians to believe that international progress was possible. But that experience, plus the difficulties in negotiating away the problem of acid rain, has made it plain that nationalistic politics have to be avoided if the global "commons" is to be protected.

The conference is being billed as strictly non-political. Those attending will be briefed on the possible effect on climate of the greenhouse phenomenon, the depletion of the ozone layer and the long-range transport of pollutants. The conference will try to "set policy goals that will adapt to, control and if possible, prevent undesirable changes in the atmosphere".

Speakers want to avoid being seen as just environmentalists preaching conservation. In pre-conference briefings, serious attention was given to the possibility that a global warming might not be a bad thing for Canada after all. Henry Hengeveld, an adviser at the Canadian Climate Centre and member of the committee organizing the conference, believes a mid-latitude warming of 2–3°C would cause a polar warming of 10°C. That would open up the north-west passage for shipping and make most of the east coast of Canada ice-free in winter.

The attempt by conference organizers to keep bilateral squabbling off the conference agenda has not, however, fully won over Canada's neighbour to the south. Fear that the conference may focus attention on US policy on acid rain means that no senior US administration official will attend.

Alun Anderson



# The pros and cons of freedom of access to human genome data

## Paris

WHAT kinds of computer programs are needed to help scientists construct a complete sequence of the 3,000 million nucleotide base pairs making up the human genome? How can the results be shared by the entire scientific community without being impeded by "commercial, political or prestigious interests"? According to the Committee on Data for Science and Technology (CODATA) which organized a two-day meeting of world experts in Paris last week, these questions "have not been sufficiently explored". But, although goodwill was abundant, the meeting also uncovered



some worries, not least the power of the media in helping or hindering governments' interest in financing human genome research.

Data-processing remains essentially a practical matter and software houses are already creating 'user-friendly' programming environments for molecular biologists. But the question of what to do with the data is less evident. "As sequences get longer", says Professor George Bell of the biophysics and theoretical biology laboratory at Los Alamos, "publication in science journals will become impractical. Nobody will read them. In a couple of years, sequences will be published only in databanks." This is why access to databanks, such as those at Los Alamos and the European Molecular Biology Laboratory, should be open to scientists everywhere — one of the meeting's resolutions.

This spirit of scientific *glasnost* also underlies the multilateral 'gentlemen's agreement' to share results, to avoid duplication and to outlaw patent applications within the human domain. But if, as Charles de Lisi, former head of genome research at the US Department of Energy, says, "private enterprise cannot do the research", governments are aware that

"transfer to the private sector will be rapid". Although the short-term rewards of human genome research will remain in the science domain — fast, low-cost automated sequencing, the availability of probes to hunt genes — the potential long-term commercial spin-off for pharmaceutical companies is incalculable.

When Boja Keil of the Pasteur Institute says that "the obstacles to progress are time and money", it is, for the moment, mostly US time and money. Japan, one of the countries best-placed to invest in human genome research, has so far only expressed interest. What is worrying some US experts is the threat of corporate takeover of US companies by wealthy Japanese banks, once the research has borne commercial fruit — a hint that political interests do not easily go away.

In Europe, politically active ecologists ('Greens') are perceived as partly responsible for the reluctance of governments to join the quest — particularly in West Germany. Aware of the potential power of the media to influence public opinion, for better or for worse, delegates at the Paris meeting resolved to seek to avoid distortion of scientific publications through popularization by overseeing journalists' interpretations.

The meeting also tackled ethical questions. Medical diagnosis — and possible treatment — of the 4,000 known single-

gene hereditary illnesses remains the most publicly acceptable motive for the research. Without the prospect of therapy however, screening is not always welcomed by potential sufferers. "But", says Bell, "if you don't know the cause you can't treat the illness."

While human genome research remains "far less expensive than a moon-shot" and with more tangible short-term rewards, Bell emphasized that the complete sequencing of a human genome is only the beginning, not the end of the search. "We say 'the' human genome, but there are perhaps as many as 10 million differences between the genomes of two parents. We don't think these are all linked to individual genes, but we do not know."

Peter Coles

■ A BILL establishing an inter-agency panel to coordinate US federal research efforts on the human genome project last week passed the Senate by an 88 to 1 margin. The Biotechnology Competitiveness Act also creates a national biotechnology policy board and advisory panel, a national centre for biotechnology information and an agricultural biotechnology panel. The act also establishes a biomedical ethics board, to review moral and ethical questions related to genome research.

The bill now goes to the House of Representatives, for hearings before three committees. Although the coming election has shortened the legislative calendar, the House may still act on the bill. Its chief Senate sponsor, Lawton Chiles (Democrat, Florida), retires this year, and has made the act a priority. J.P.

## Early retirements in Academy's *perestroika*

### London

SIXTEEN officials of the Soviet Academy of Sciences have resigned in advance of the expiry of their term of office in 1991. They include six academic secretaries of departments of the Academy and 10 members of its ruling presidium. The resignations were hailed by the government newspaper *Izvestiya* as an example of Mr Gorbachev's policy of "rejuvenation of cadres", that is, of the retirement of holders of administrative offices to make way for younger people.

Just when the retirement took place is not clear. *Izvestiya* reported it on 10 June, with a note that elections to fill the vacant offices would take place before 20 June. In fact, said *Izvestiya*, one such election — for the secretaryship of the Department of General and Technical Chemistry — had already taken place.

The head of the Academy's personnel department told *Izvestiya* that the resignation of so many high-ranking officials, although unprecedented, was simply part of the normal process of "changing the generations".

Nevertheless, it is clear that many

members of the Academy are opposed to Mr Gorbachev's "rejuvenation" policy. Last autumn, when the first round of elections came due for vacancies created by the compulsory retirement of superannuated academicians, a significant number of the candidates proposed turned out to be over the new age-limit of 60. And, as *Izvestiya* noted, the Academy still has 20 directors of institutes and 160 heads of divisions who are well past retirement age.

Superannuated officials who retire from office can still serve as "advisers" to the presidium, and many scientists who have retired in this manner have expressed their satisfaction at being relieved of tedious administrative duties. But not all, it seems, are willing to quit. The 16 eminent persons (including a former president of the Academy, Dr Anatolii Aleksandrov) who have retired *en masse* have, said *Izvestiya*, received official commendations for their "outstanding services to science and the organization of science". The tone of the *Izvestiya* report suggests that their good example in retiring is not the least of these "services".

Vera Rich

# UK research councils continue to squabble over biology resources

## London

BRITAIN'S Science and Engineering Research Council (SERC) has launched a vigorous defence of its role in biological research, raising the temperature of the debate about how best to tackle overlapping interests between the four natural science research councils.

The question about the division of biology between the councils has surfaced during evidence to the House of Lords Select Committee on Science and Technology, which is investigating agriculture and food research in Britain. Conflicting views on the perceived problem of the imperfect distribution of biology resources have prompted the Advisory Board for the Research Councils to set up a committee to examine the present

system and recommend any changes deemed necessary. Of the various options suggested so far, most would result in SERC being deprived of its role in biological research.

That SERC feels under siege is clear, and the council's public relations staff have been working overtime to ensure that its side of the story is heard. SERC's annual expenditure on biology is around £23 million, which is spent wholly in higher education institutions and which supports around 1,700 research students. In evidence to the Lords' committee two weeks ago, SERC chairman Bill Mitchell made it clear that his council would not relinquish its biology interests without a fight.

The nub of Mitchell's argument is that biology must strengthen its links with the other core sciences and that manpower training is paramount; SERC is best placed to achieve these ends. Mitchell is thus firmly opposed to the establishment of a single non-medical biological research council, an option that has been widely canvassed. Such a move would set an unhealthy precedent for discipline-based research councils, he says.

Mitchell cites SERC's synchrotron radiation source at Daresbury as an example of a venture which he suggests "would not have got off the ground so easily" had there been a single biology council. While on the topic of synchrotrons, Mitchell took the opportunity to mention that, despite being asked, the Medical Research Council (which is eyeing SERC's biotechnology) has not made a contribution to the European Synchrotron Radiation Facility at Grenoble, with Britain's contribution being shouldered solely "and with great difficulty" by SERC.

The Royal Society is the latest body to give support to the concept of a non-medical biology research council, outlined in written evidence to the Lords committee. While advocating that SERC's biology be relinquished to the new council, the society says that "the benefits to biology of direct contacts with the other disciplines [chemistry, physics and engineering], and conversely the benefits to them of links with biology, are substantial and growing, and any new administrative structures would have to ensure these beneficial interactions were maintained and enhanced".

The society, says Mitchell, apparently "wants to have its cake and eat it". The concept is fuzzy and, furthermore, despite being a fellow of the society, he was not shown the evidence before it was submitted.

Simon Hadlington

## Successful test firing

### Washington

A two-minute test firing last week of the redesigned solid-fuel booster rocket for the space shuttle seems to have been a success. A final verdict will have to wait until engineers from the National Aeronautics and Space Administration (NASA) and Morton Thiokol disassemble the rocket casing and inspect the segment joints.

One further booster test remains before the shuttle can be declared operational. In that test, now scheduled for the end of July, engineers will introduce deliberate flaws in the rocket joints to determine if their new design will prevent the type of exhaust leak through the O-ring seals that caused the Challenger accident in 1986.

The resumption of space shuttle operations is now scheduled for the end of August.

J.P.

## Projects for Max Planck

### Heidelberg

THE Max Planck Society announced at its annual meeting on 8 June that, given sufficient outside funding, two new projects are ready for approval: an informatics institute in Saarbrücken, and an eight-year project in cognitive anthropology in West Berlin. But funds must be proffered by the Saarland and West Berlin before the projects can begin. Spokesman Michael Globig said the society would try to arrange this support before the 10 November meeting of the Max Planck Society senate, which could then officially establish the two projects.

At the same time, president Heinz Staab announced that the society will establish a research group for retrovirology in Göttingen. The group will carry out three projects related to AIDS research in collaboration with the German Primate Centre.

S.D.

## New Soviet telescope

### London

THE world's largest millimetre-band radio telescope is now under construction on the Suffa plateau in Uzbekistan, *Izvestiya* reported on 31 May. Quoting V. Shlykh, section head at the Space Research Institute of the Soviet Academy of Sciences, the paper said that the 70-m Suffa telescope will work in conjunction with an orbiting radio telescope to study astronomical objects and to determine with a high degree of accuracy the Earth's continental drift and the position of the poles. It will also be used for collaborative studies with the two other large radio telescopes in this wave-band — the 30-m radio telescope at Pico Veleta (Spain) and the 45-m instrument at Nobeyama (Japan). The most remarkable feature of the Suffa facility, *Izvestiya* said, will be the high accuracy of the main dish, which, it is estimated, will deviate from true parabolic form by not more than 0.07 mm.

V.R.

## First victory over tobacco companies

### Washington

LEGAL history was made last week when, for the first time, a tobacco company was judged partially liable for the death from cancer of a smoker. A federal judge in New Jersey ordered the Liggett Group to pay the widower of Rose Cipollone \$400,000 because, before 1963, the company had advertised its cigarettes as being safe. In one advertising campaign for L&M cigarettes the company went so far as to declare that its cigarettes were "just what the doctor ordered".

The verdict is not expected to open a floodgate of lawsuits over deaths that may be linked to cigarettes. The jury determined that Cipollone was 80 per cent responsible for her own death. The jury also disagreed with the claim by Antonio Cipollone's lawyers that there was an industry-wide conspiracy to withhold information about the health risks of smoking. Industry lawyers say the relatively small size of the award, and the decision to leave intact the tobacco companies' traditional defence that smokers are responsible for their own actions, will discourage future legal claims.

But consumer activist groups disagree, arguing that much of the legal groundwork in the Cipollone case will make future lawsuits easier to pursue. Many tobacco company documents entered the public domain during the trial, and can be used again. Other documents, not permitted as evidence in this case, may yet surface in other actions. Perhaps even more significantly, the Cipollone decision seems to have encouraged Congress to look more closely at federal regulation of the tobacco industry.

Joseph Palca



# German science in danger as hope for big increase fades

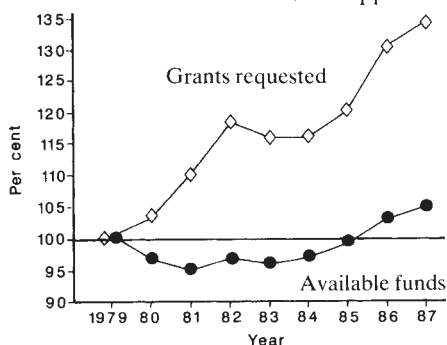
**Bonn/Heidelberg**

BEGGING for money is not Hubert Markl's favourite activity. But Markl, the president of the Deutsche Forschungsgemeinschaft (DFG), went before a group of West German parliamentarians in Bonn on 8 June to plead his case for a 5 per cent increase in the DFG budget for 1989.

The increase is especially crucial in view of the large number of university graduates expected in the coming years, a result of the postwar birth bulge.

Unlike some politicians, Markl views the abundance of new graduates as a "gold reserve". But he warned the Bundestag that if graduates who want to enter basic research are not supported right away, they will be lost to the universities.

The DFG, which funds basic research in West German universities, is supported



by the federal and *Länder* (state) governments, both of which must agree on the budget. The finance ministers of the *Länder* proposed an increase of only 2.5 per cent to the annual DFG budget of DM1,100 million, despite Markl's visits to the political chiefs of most of the eleven *Länder* within the past year.

But the *Länder* may be persuaded to increase their contribution if the federal government will go along. Delicate negotiations between Education Minister Jürgen Möllemann and Finance Minister Gerhard Stoltenberg may yield a higher federal contribution. But if this attempt fails, the DFG will take its case before the West German cabinet in early July. This, according to sources within the DFG, will be the best chance for an increase.

Chancellor Helmut Kohl's centre-right coalition is now busy trying to reach a compromise on larger-scale issues such as welfare, tax and pension reform as well as the research budget before the parliamentary recess begins on 8 July.

Even a 5 per cent increase would not come close to maintaining the ratio of DFG grants to applications, said Markl. The money requested in grant applications has grown by more than 10 per cent a year while the budget has remained almost constant in real terms (see graph).

There is particularly strong competition for grants in biomedical and high-technology areas of research, areas which are economically important for West Germany.

At the same time, he reminded the Bundestag members that several thousand more university graduates will be seeking doctoral and postdoctoral support from the DFG for each of the next several years.

Markl said that the "bare minimum" at which the DFG can continue to fund all the superior grant applications for both graduate stipends and project support is a 5 per cent increase in 1988, followed by 5.5 per cent in each of the next two years. The DFG budget had already fallen short of those expectations by the time of his statement, having received 3 per cent for 1988.

If the DFG is granted 5 per cent in 1989, "we will put all of it into supporting young researchers", said Markl. The increase would "send a sign to the students" and encourage them to continue applying for support. The influential Wissenschaftsrat and Kultusministerkonferenz as well as the Education Ministry support this rationale, he said.

Markl mentioned the example set in Switzerland, where the Nationalfond, the DFG's counterpart, has been granted an annual budget increase of 3.8 per cent over the next three years because of a similar demographic bulge.

The Max Planck Society (MPG), which has received slightly larger increases than the DFG in recent years, is having budget troubles too. MPG president Heinz Staab announced at the society's annual conference that a resolution passed by the Bundestag budget committee last year may reduce the MPG budget for 1988. In addition, a long-awaited equipment replacement programme was postponed again until at least 1989.

Staab called the threatened cuts "incomprehensible" in the light of the unanimous support that the federal and *Länder* governments had declared for the MPG last year.

A representative of industry, Hermann Freudenberg, who was present on 8 June, implored Bundestag members to grant the DFG's request. The DFG budget is tiny, he said, in comparison to the DM41,000 million spent in 1987 on public support of research and development. But Bundestag budget committee member Karl Diller (Social Democrat) painted a gloomy picture of the prospects of squeezing out a few extra million for the DFG. Mercy, if it arrives, will have to come from the top.

Steven Dickman

# Ariane-4 launched successfully

**Paris**

EUROPE'S new Ariane-4 commercial rocket launcher successfully put its payload of three satellites into orbit on 15 June, only six minutes behind schedule (see *Nature* 333, 591; 1988). This demonstration flight, which initially was to carry ballast only, not only helps Arianespace — the European consortium operating Ariane — to reduce its backlog of orders. It was also an essential reinforcement for Europe's determination to hold onto its 50 per cent share of world commercial launches in the face of the United States' imminent comeback, the entry of the Soviet Union into the market and competition from the Chinese and Japanese.

The US presence in the commercial launch market was suspended in 1986 when the manned Challenger shuttle tragically exploded shortly after lift-off. Since then, the United States has put only military payloads into space. But the technological experience gained will not be wasted, as Martin Marietta's Titan 3 commercial launcher — Ariane-4's most serious competitor — shares many of the components used in the smaller, military rockets. McDonnell Douglas is also building 20 less-powerful Delta launchers for the US Air Force. Although not a direct competitor, each Delta-2 rocket can separately carry one of the satellites Ariane-4 will be able to launch in pairs or threes. Ariane-4's multiple payload capacity is an important selling point, necessary to keep costs down and to meet launch deadlines.

Having offloaded development costs onto the defence budget, the United States will undoubtedly be able to offer its launches at cut price — to the chagrin of Arianespace and the European Space Agency (ESA), which have always depended on civil and private-sector investment. What ESA calls the "formidable launch capacity" of Soviet Proton launchers is also being offered to the world commercial market for the first time, again following years of state military investment in development.

If the low cost of Chinese CZ-3 (Long March 3) launches cannot easily be countered by the Europeans, the Japanese still have nothing to match Ariane-4's 4,200 kg payload capacity. Trends in satellite technology were not well forecast by Japanese constructors, so that the H1 launcher is already outclassed.

The success of Ariane-4 is also a shot in the arm for France, which provided nearly 60 per cent of the development finance. In the face of recurrent doubts from other European partners, France has consistently placed a high priority on its — and Europe's — presence in space. Peter Coles



## Japanese students protest at plans for graduate school

Tokyo

"CRUSH the graduate school reform scheme" screamed a poster at Tokyo University's main gate as 13,000 students from Japan's leading university went on strike earlier this month for the first time in eight years. The students, who boycotted classes on 7 June, are protesting at plans to strengthen the university's graduate school, suspecting that it is a grand strategy to tighten links with industry and increase the 'elitism' of the university. But reformers in the Faculty of Science, where the plan originated, say the changes are intended to create a more open and international university.

The main changes proposed are to establish a new campus for the graduate school in Chiba Prefecture, on the outskirts of Tokyo, and to fuse the undergraduate and graduate systems by establishing a 6-year course for a master's degree (which entails the elimination of the entrance examination to the graduate school for Tokyo's own students).

But each faculty has its own ideas about how the new graduate school should operate. Professor Akiyoshi Wada, who was appointed chairman of the science faculty's committee for reform in April, says that supporters of reform have been putting "the cart before the horse" by demanding more money and facilities for the graduate school without first defining their objectives. Under the science faculty's plan, the new campus in Chiba would, if established carry out 5-7 year projects in new fields, such as space and

life science, while research in more traditional areas would continue at the main campus in Tokyo. The faculty also hopes to establish a new post for young researchers to replace that of 'research assistant' (*joshu*).

The *joshu* system came under public scrutiny last year when a research assistant at Hiroshima University murdered his professor for passing him over for promotion after 17 years of service. A few months later, Nobel prizewinner Susumu Tonegawa criticized the system, which he says stifles young researchers under the control of autocratic professors.

Wada says the "Tonegawa typhoon" is helping the push for the new post, which he sees as being a non-tenured research position somewhere between a postdoctoral fellow and an assistant professor. Wada also hopes to establish more positions for foreign students, postdoctoral fellows, visiting professors and permanent faculty. But entrance examinations to the graduate school for non-Tokyo University students will have to be retained, at least until other universities in Japan adopt the same system, Wada says.

The Faculty of Engineering, on the other hand, which also has advanced plans for reform, seems to have its eyes on more domestic matters. The faculty hopes to strengthen links with industry through graduate school reform and plans to set up research institutes in collaboration with industry. It is these plans which have provoked vehement student protest.

David Swinbanks

## Caltech graduate course will change thinking on computers

Pasadena

AN exciting new graduate course linking computation and brain function seems to have made a mark on intending students at the California Institute of Technology (Caltech) after only its first year.

Formally known as Computation and Neural Systems, the course, leading to PhD by thesis, recruits both engineers and biologists. Christoph Koch, one of the directors of the course, argues that understanding the design principles on which the brain works can contribute to the design of intelligent computers. Carver Mead, an engineer building analogue chips based on structures such as the mammalian retina, says that there may be much to be learned about component interconnection from the compromises reached during natural evolution.

Reciprocally, neuroscientists appear

rapidly to have appreciated the power of parallel computing techniques for modelling experimental systems, for which reason the words neural networks are on every tongue.

The new course is a joint initiative of the three divisions of Biology, Engineering and applied sciences and Physics, mathematics and astronomy. One major goal is the encouragement of interdisciplinary research projects, although the immediate incentive for founding a formal course seems to have been the large numbers of engineering students enrolling in biology graduate programmes.

Now, student demand seems to have taken Caltech by surprise. James Bower, joint director of the programme, says that he had planned to take only five students in the past academic year, but that "we could not choose between the top ten". By

## Australia's Labor party looks right

Sydney

AUSTRALIA'S Labor party, in power since 1984, last week moved to sever several ties to its more socialist past. Speaking in Hobart, at the party's biannual conference, Prime Minister Bob Hawke backed changes in policy that would relax constraints on uranium sales. And in a historic decision, the party voted to abandon the commitment to free tertiary education.

The Labor party's policy has been to try for an eventual end to all uranium mining in Australia. Its aim has been to try to restrict the spread of nuclear weapons by banning the export of uranium from Australia. All shipments of yellow cake (unprocessed uranium) to France were banned as a protest against France's nuclear tests in the South Pacific. The policy took its first hard blows in 1986 when for economic reasons the government decided to resume uranium sales. At the Hobart conference, Senator John Button, the Minister for Industry, Trade, and Commerce, suggested that uranium might be enriched in Australia. France makes A\$250 million a year by enriching Australian uranium.

Hawke claimed that the Labor party never had an anti-uranium position, but simply a position of "accommodation to changing realities". The party's left wing does not agree and is certain to fight hard. But right and centre-left factions of the party joined forces to pass a motion that would deflect opposition by referring a decision to a postal ballot of conference delegates.

The decision to abandon free tertiary education represents a complete reversal of policies implemented by a previous Labor government in 1974. The new policy clears the way for the Minister for Employment, Education and Training, John Dawkins, to proceed with the implementation of the "learn now, pay later" tax recommended by the Wran committee (see *Nature* 333, 103: 1988).

Charles Morgan

contrast, there seems to have been a marked decline in applicants for graduate programmes with a molecular orientation in the past two years.

Caltech's new course also seems to have attracted an unusual (for Caltech) proportion of women, as well as support from industry. Much the same trends have appeared at the University of Pennsylvania, where Edward Pugh, professor in the psychology department, says that students are asking what molecular neurobiology has to say about processes such as perception and cognition. Pugh believes this trend may signal a switch in neurobiology towards the systems rather than the molecular approach to problems.

Jennifer Altman

# Privatization one of the 'cures' being considered for NIH

## Washington

A US Institute of Medicine panel last week took public testimony on ways in which the intramural programme at the National Institutes of Health (NIH) might be improved.

Nobody denies that NIH are facing difficulties. The most frequently cited problem is money. As federal employees, NIH scientists face salary and benefit limits that do not exist in industry or at academic institutions. But even if salaries could be made more competitive, other issues confront NIH researchers.

It is difficult, for example, to build large research teams at NIH, because personnel numbers are relatively inflexible and laboratory space is at a premium. Travel is also more difficult, as researchers' travel plans must also conform to government-approved standards.

Although these problems are not new, headlines last year in the *Washington Post* saying that National Cancer Institute researcher Robert Gallo was considering leaving NIH focused attention on them. Gallo is widely respected both in the White House and on Capitol Hill for his work on AIDS and the prospect of his departure convinced some that the time had come for action.

One plan considered by the White House Office of Management and Budget would have transferred some of the intramural programme to a private foundation that might run the facility as a research university. Details of the plan were leaked to the *New York Times*, prompting an outcry that the White House was trying to sell one of the "jewels of the crown", something OMB vigorously denied (see *Nature* 330 680; 1987). Instead, OMB argued that it was merely looking into ways of preventing any further deterioration of NIH's lustre.

The Institute of Medicine's panel is not, as panel chairman Harold Shapiro, president of Princeton University, took pains to point out, looking solely into the question of privatization.

Shapiro emphasized that the panel would weigh many possible options for improving the intramural programme. One plan, already being considered by Congress, would create a Senior Biomedical Research Service, with "enhanced compensation rates".

But George Cahill, vice-president for scientific training and development at the Howard Hughes Medical Institute, says that salary problems are not limited to senior researchers. Mid-level scientists are also finding it tempting to leave for more lucrative pastures.

Cahill, like others testifying before the

panel, agreed that NIH are still an outstanding research centre that will attract top scientific talent. Not only are scientists free from teaching duties at NIH, but they also receive research support directly from their parent institute, an extremely

important attraction for researchers anxious to avoid the grant-writing grind.

The institute panel will continue to hear testimony throughout this month, and plans to release its report in October, an extremely short turn-around time. Indeed, some potential witnesses were annoyed at being given only one week's notice of last week's hearing. The panel will accept written comments during the rest of this month.

Joseph Palca

# Drug use a problem at US weapons laboratory?

## Berkeley

DRUG abuse may be widespread at the US government laboratory that conducts research crucial for the next generation of nuclear weapons. According to a congressional report issued last week, an investigation at Lawrence Livermore National Laboratory (LLNL) into illegal drug use by employees, code-named Operation Snowstorm, was apparently more successful than LLNL officials had anticipated. It was called off before the results became too embarrassing, according to the report issued by the House of Representatives energy and commerce subcommittee on oversight and investigations. The report also accuses Department of Energy (DoE) officials of hiding the investigation from subcommittee members.

Operation Snowstorm began in January 1986, when allegations surfaced of drug use by laboratory employees. An undercover officer posing as a truck driver identified 11 possible drug dealers and 24 users inside the laboratory. During the investi-

gation, he collected information suggesting that more than 100 laboratory employees, including scientists and staff with high security clearances, were using, buying or selling drugs on the job. LLNL officials called off the operation in September 1986, just days before the officer received a security clearance that would have allowed him to investigate the most sensitive areas of the laboratory.

Laboratory officials say the investigation had served its purpose and was called off to begin prosecution of those employees about whom incriminating evidence had been gathered.

Six members of laboratory support staff have been arrested on drug charges, and another 10 were forced to resign. According to a laboratory spokesman, there was insufficient evidence to pursue the remaining 100 or more suspect employees.

But members of the subcommittee as well as the investigators involved in the project accuse DoE and LLNL officials of stifling the investigation, when it had only begun to scratch the surface.

One investigator, Tim Mitchell, told a congressional hearing last week that he was warned not to investigate a laboratory chemist with high security clearance, Ronald K. Stump. Mitchell also said that \$11,000 worth of precious metals had been checked out from the laboratory in his name, and not recovered.

During subcommittee hearings last week on the drug charges, several laboratory investigators who took part in Operation Snowstorm quoted John Hunt, head of security at LLNL, as saying that if the investigation were to continue, "we would have to arrest 20 per cent of the lab". But Hunt denies having made the comment, according to a laboratory spokesman. When Operation Snowstorm was prematurely cancelled, the investigators complained, and each has subsequently been demoted.

Not convinced that the problem has yet been cleared up, the subcommittee has asked LLNL director John Nuckolls to submit a written proposal outlining further action he plans to take.

Marcia Barinaga

# Livermore clean-up

## Berkeley

JUST one week after a nationwide study criticized US Department of Energy (DoE) laboratories for mismanagement of radioactive waste (see *Nature* 333, 591; 1988), the DoE's Lawrence Livermore National Laboratory announced plans to build an on-site treatment plant for radioactive and toxic wastes. A laboratory spokesman said the announcement was not a response to the study by the New York-based Radioactive Waste Campaign, but was the culmination of several years of planning for the \$41 million facility.

The treatment plant, scheduled for completion in 1992, will allow on-site volume-reduction and detoxification of 90 per cent of the laboratory's toxic and radioactive waste, compared to current on-site treatment of only 10 per cent.

The laboratory is also beginning a \$60 million clean-up of a toxic burial site, where leaky drums have contaminated groundwater.

Marcia Barinaga



## New areas in AIDS research

*Stockholm*

A VARIETY of pleas marked the closing stages of the IVth International Conference on AIDS. Loudest among them were calls for more basic virology, more sex research, more attention to other sexually transmitted diseases and an intensified search for an animal model (see page 699).

"There is a future in turning back to the past", was how David Baltimore, director of the Whitehead Institute for Medical Research, couched his plea. We have learnt a great deal about the sequence variations of human immunodeficiency virus (HIV) and its genes, he said, because that was what was most easy to explore when the virus was discovered. But relatively little is known of the basic life cycle of the virus.

It is necessary to go back to a basic virological study of HIV to try and find chinks in its armour, Baltimore argued. "We are too concerned with drugs and vaccines", given that it is now clear they are not easy to come by. More needs to be learnt about regulation of the life cycle of HIV.

King Holmes, of Harborview Medical Center in Seattle, pleaded for more to be spent on the study of sexually transmitted diseases that cause genital ulcers and for the control of chlamydial infections because both are strongly implicated as risk factors for HIV transmission. It is disastrous, he said, that the control of chlamydial infections has been stalled for the past four years because funds have been diverted to AIDS research. There is little cause for complacency in the slow growth of AIDS associated with heterosexual transmission of HIV in view of the alarming spread of sexually transmitted diseases in some population groups.

While calling for much more research on human sexual behaviour, in part to provide the data necessary to model the spread of HIV, John Gagnon of Princeton University warned that many poorly designed and inadequately performed sex surveys lead to "blind alleys, out of which people must publish their way". He lamented the fact that it has taken AIDS to stimulate the need for surveys; that they are necessary is evident by the continued reliance on the Kinsey Report.

A number of adequate studies of sexual habits in the United States, Europe, Africa and South America are now in the planning stage, Gagnon said. He welcomed the controversial involvement of homosexuals and prostitutes in such studies, for they may best help to define the questions that should be asked.

Peter Newmark

## Plans for altered lymphocyte release in humans

*Washington*

Two researchers at the US National Institutes of Health (NIH) are seeking permission to perform a controversial experiment requiring the administration of genetically-engineered cells to people. If authorized, it will be the first officially-sanctioned experiment in which human cells altered by recombinant DNA techniques are returned to the body.

The experiment, proposed by Steven A. Rosenberg of the National Cancer Institute (NCI) and W. French Anderson of the National Heart, Lung and Blood Institute, will track lymphocytes which have been activated to fight cancer as part of the tumour-infiltrating lymphocyte (TIL) therapy developed by Rosenberg.

In this technique, so far effective only in patients with skin and renal cancer, lymphocytes which have already proven their

anticancer ability by invading a tumour are selectively stimulated to grow by excising the tumour and treating it with interleukin-2 (IL-2). The purified tumour-invading lymphocytes are then returned to the patient with additional IL-2, in the hope that the activated lymphocytes will attack the remaining cancerous tissue.

There is now no way to trace the movements of the reintroduced lymphocytes to prove that the strategy is working, because the half-lives of the radioactive labels which are safe for use in people are too short. Rosenberg and Anderson plan to introduce the gene for neomycin resistance into the activated lymphocytes before they are administered to the patient in order to follow their action in TIL therapy.

By strict definition, the proposed experiment is not gene therapy, because it will not replace a gene whose absence or defect is causing disease. Rather, the experiment can be likened to environmental releases of recombinant organisms where genes which allow the identification of the altered organisms are introduced instead of a functional genes producing a desired product.

The bulk of Anderson's research has been on developing a therapy for infants with severe combined immune deficiency (SCID) who lack a functional gene for the basic enzyme adenosine deaminase (ADA). Anderson and his coworkers are laying the groundwork to allow them to restore the normal operations of SCID patients' immune systems by inserting the ADA gene into cells from their bone marrow using a retroviral vector.

Before the marker experiment outlined by Rosenberg and Anderson can proceed, it must be approved by the human experimentation board at the NCI, the Gene Therapy Working Group of the Recombinant DNA Advisory Committee, and several other bodies. If the experiment is approved and proves successful, the next step would be to engineer the lymphocytes to produce larger quantities of their own IL-2, removing the necessity for follow-up IL-2 injections which cause side-effects.

The only other experiments which have involved treating humans with genetically-engineered cells were performed in 1980 by Martin J. Cline of the University of California at Los Angeles. Cline attempted unsuccessfully to insert haemoglobin genes into patients in Italy and Israel to cure them of beta-thalassaemia. Because Cline conducted the experiments without undergoing the review process to weigh the risks and benefits of the therapy, he is no longer eligible to receive federal funds for his research.

Carol Ezzell

## Drastic measures proposed in India

*New Delhi*

SEXUAL intercourse with foreign nationals will be made a punishable offence under legislation that the Indian Council of Medical Research (ICMR) has asked the Health Ministry to introduce parliament in February 1989. The controversial proposal is one of ICMR's strategies to check the spread of AIDS in India.

According to Dr A.S. Paintal, director-general of ICMR, the proposal was favourably received by the Ministry of Health and other relevant ministries. But the Law Ministry says it violates the constitutional right of privacy. ICMR, which says the constitution must be amended if possible, plans to elicit public opinion through nationwide debates and seminars. A national conference is scheduled for January 1989 and its recommendation will be presented to the parliament the following month.

Paintal, who believes that AIDS is an imported disease, is personally behind this move. He fears that there is a real danger of AIDS flaring up in India through sexual contacts with visiting foreigners. A law banning such contacts is seen as a preventive measure. It will not harass the foreigners, but Indians caught in the act will pay a fine of Rs20,000 and will spend three months in gaol.

One question Paintal has not answered is whether it is practical to enforce the act without an army of inspectors peeping into bedrooms through keyholes. ICMR admits that catching offenders will pose a problem but hopes the mere existence of the law would act as a deterrent.

K.S. Jayaraman



## Science teaching in the UK

SIR—M.H. Dodson, in his letter entitled "UK science teaching" (*Nature* 333, 9; 1988) points out that the changes towards balanced science courses have "apparently been approved" by professional bodies with little thought for the implications.

The Institute of Biology has not added its name to a recently published list of supporters of balanced science courses because of its reservations about lack of resources and training, hurriedly prepared syllabuses and possible inadequate preparation of pupils for sixth-form study. The institute has pointed out many times that, unless a course is modular, teachers will be required to teach outside their applications, and until resources are provided for in-service training, standards may drop.

Recruitment to the teaching profession has dropped alarmingly over recent years in all science subjects in the United Kingdom, including biology. This is, in no small measure, due to the tremendous number of changes teachers are expected to cope with, imposed on them by a frantic and didactic government department.

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SIR—M. H. Dodson (*Nature* 333, 9; 1988), protesting at the damaging curriculum in British schools, fails to recognize the advantages of 'integrated science' or

'balanced science' for 14–16 year olds. He notes that integrated science is the equivalent, for timetabling purposes, of just two individual subjects and refers to the pressure upon specialist teachers of physics, chemistry and biology to teach outside their own specialities.

But at present fewer than 10 per cent of pupils take three separate sciences in the fourth and fifth years. The introduction of double-certificated science will mean an increase for most children in the amount of science they do at school. On his second point, most schools introducing integrated science divide up the syllabus into its traditional parts, arranging for physics to be taught by a physicist and so on. But physics teachers, and increasingly specialist chemists too, are sufficiently rare in most schools for there to be no chance of their being required to "labour over the microscopy of living cells" as he fears.

Where I do agree with Dobson is that the introduction of such syllabuses, and GCSE in general, has profound consequences for the factual and skills content of A-level subjects. Indeed, the major examination boards are currently producing revised A-level syllabuses. As is widely recognized, British science A levels are very difficult. Many teachers, and the vast majority of students, would welcome changes leading to a broadening of the sixth-form curriculum. Britain desperately needs more science graduates, and does itself no service by making science A levels accessible to only a very small minority of its 16–19-year olds.

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## Just good friends?

SIR—Your report on the impact of the review on geology at Imperial College (*Nature* 327, 489; 1988) refers to a "bitter dispute" between the chairman of the University Grants Committee (UGC) and myself. I assure you that there is none; we are on the best of terms. I see the approach taken by UGC over the past few years as a major advance in tackling the fiendishly difficult problem of how to ensure an equitable distribution of unhappily inadequate resources.

My concern is simply that the application of criteria which are wholly appropriate for the assessment of pure science are likely to give the wrong answer when used with respect to an applied science department. The opposite — judging pure science by an early assessment of its potential utility — is of course equally inappropriate.

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SIR—As practising science teachers we were surprised at the views expressed by M.H. Dodson (*Nature* 333, 9; 1988) about the suggested changes in the science curriculum. In particular, Dodson promulgates three serious myths about the present state of science education in Britain.

First, he says that the education that students receive at school is merely an apprenticeship for the real education that they will receive once they reach university. As the vast majority of our students never go to university, this surely represents a major distortion of their curriculum. University entrance requirements have distorted the educational system for too long.

Second, he says that the present standards of science education in Britain are high. By any criterion this is simply not true. We send fewer students on to higher education, particularly in science and engineering, than any of our major industrial competitors. A large proportion of

students drop one or more science subjects before the age of 15. In particular girls drop physical science subjects. The suggested changes in the science curriculum are designed to remedy this appalling state of affairs.

Third, he says the proposed changes suggest balanced science courses not balanced science teachers. While we agree that much in-service training will be needed, we suggest a more appropriate model would be specialist science teachers working together in teams to deliver a more relevant science education in a more relevant way. Incidentally, we both find teaching outside our subject specialities a particularly rewarding experience.

Finally, the only way that we can possibly "broaden our notoriously narrow sixth form curriculum" is to adopt a balanced approach pre-16. If the price that has to be paid for providing a better science education for all our students is that universities have to alter their current teaching practices, then so be it.

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## The data explosion

SIR—John Maddox (*Nature* 333, 11; 1988) highlights the thorny issue of data for data's sake versus the search for unifying hypotheses. I believe that the lack of such hypotheses, particularly common in the biological sciences, can be laid in no small measure at the door of journals such as *Nature*. These publications do not have a section devoted to theoretical papers dealing with the life sciences. Indeed, if an author has the temerity to submit such work for consideration, it is invariably returned with the comment that there are insufficient experimental data, or that it is "not of wide enough interest". Naturally it is much simpler to publish data only, as the guidelines for acceptance are more obvious. On the other hand, it is clearly more difficult to establish such standards for accepting theoretical papers. Nevertheless, publishing such work may serve a more important function in the long run than the reams of data at present filling most space in leading scientific journals. Until journals such as *Nature* have a regular section devoted to theoretical aspects, particularly in biology, they are playing truant to their responsibility.

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# Receding hopes of AIDS vaccines

*Prospects that there will soon be an effective vaccine against HIV are diminishing. In the absence of a good animal model of AIDS, direct human trials of candidate vaccines are inevitable.*

## Stockholm

It would have been extraordinary if the first shots at making an AIDS vaccine had been effective, so there need not be too much despondency now that they seem likely to fail. Not that they have failed yet in human beings; indeed, the trials have only just got under way. But the lack of success of initial trials in chimpanzees and rhesus monkeys cast a dark shadow over the IVth International Conference on AIDS, held here last week.

Pessimism tends to take over when someone like Gordon Ada, who is currently with the World Health Organization and has a wealth of experience in viral vaccines, says that the problems of producing a vaccine against the human immunodeficiency virus (HIV) are greater than for any other virus. Justifying that statement at the end of the conference, Ada listed a string of reasons. One is that HIV is notoriously variable. No two isolates are identical. Each isolate contains many variants. Variation may be HIV's way of escaping destruction by the immune system, and may yield variants of increasing pathogenicity and preferences for one tissue over another in the course of disease. A successful vaccine would have to be able to deal with a wide and shifting variety of strains.

A second serious problem is that the virus ducks for cover shortly after infection. That is to say, once HIV has infected a cell, its RNA genome is transcribed into DNA by reverse transcription, which then becomes integrated into the chromosomal DNA of the infected cell. An effective vaccine would have to prevent the virus from infecting any cells, for no vaccine can attack the integrated DNA. Viruses subsequently made under the instructions of that DNA will be susceptible to attack if they leave the shelter of the cell. But there is growing evidence that some cells may transmit their HIVs directly to other cells, without releasing them. To make matters worse, it may be that the original infecting virus is largely within cells in the blood, semen or vaginal fluids, rather than free, and is also transmitted from cell to cell. In that case a vaccine would never get sight of HIV in the first place.

Had all this been known three years ago, when the first candidate vaccines were on the drawing board, it might have influenced their design. As it was they were hurriedly designed from what was most available at the time. In essence, that

meant the envelope protein of the virus. This forms the external surface of the virus and therefore is most easily seen by the immune system. It is also the protein that varies most between strains.

At the latest count no chimpanzee vaccinated with the envelope protein has been protected against infection by HIV. This is true whether the vaccine is made from the protein itself, or whether it consists of vaccinia virus, the smallpox vaccine, genetically engineered to contain the gene for HIV's envelope protein. The potential advantage of vaccines of this type is that live viruses activate parts of the immune system that proteins alone cannot reach. In doing so, they stimulate the production of immune cells that can recognize and kill virus-infected cells, which may well be a more important defence system than the production of antibodies.

Because of the shortage of chimpanzees, it has not been possible to ring the various changes that might turn the initial failures into success. What makes it questionable whether any easy change in the vaccine will make a difference is the failure of a trial of passive immunization described by Jorg Eichberg of the Southwest Foundation for Medical Research in Texas. Chimpanzees were infused with large quantities of immunoglobulin purified from the blood of AIDS patients. As a result they were briefly extremely well armed not only with antibodies against a range of HIV proteins, but also antibodies that neutralize the virus and antibodies that prevent the virus from fusing cells together. Yet, when challenged 24 hours later with HIV, they were infected.

Despite, or perhaps because of, these failures, four starts on vaccinating human beings have begun. One uses envelope protein alone. Made by MicroGeneSys, the protein has been given in four different doses to homosexual volunteers, some of whom received a booster shot one month later. The higher doses sometimes, but not reliably, induced antibody production and a positive lymphocyte blast transformation test. A yet higher dose, 180 micrograms, will now be tested.

Another vaccine, described recently in *Nature* (322, 728; 1988) uses both an engineered vaccinia virus and cells taken from the vaccinated subject infected with virus, killed and given back by slow infusion. Daniel Zagury, who has used himself as a guinea pig for this complex procedure, is now seeking a simplified

version. In a third trial, with very tentative beginnings, two patients in London have been given an (anti-idiotypic) antibody designed to prevent infection by HIV.

Master-minded by Jonas Salk, the fourth trial involves giving killed HIV to people already infected with the virus in an attempt to boost their immune reaction against it. The virus, which is killed by gamma irradiation and then purified with the loss of envelope protein, was first given to nine people with AIDS related complex — the condition that precedes full-blown AIDS — last November. Salk reported just a hint of benefit in the results from this group so far. A second group of nine people joined the trial in March and 54 matched pairs of infected but asymptomatic volunteers have been recruited for the third stage.

The killed virus preparation has also been given to one uninfected and two HIV-1 infected chimpanzees, but they have yet to be tested for resistance to infection or reinfection. That humans greatly outnumber chimpanzees in the trials is a measure of the shortage of the latter. Moreover, chimpanzees do not develop AIDS after infection. For both reasons the hunt for an alternative animal model is intense. Since some simian immunodeficiency viruses (SIV) will produce AIDS in rhesus monkeys, this is a promising, if inexact model.

Unfortunately, the first attempt to vaccinate rhesus monkeys against SIV infection, using an inactivated SIV vaccine at the New England Primate Center, has failed. Another hope is that HIV-2, the predominant AIDS virus in western Africa, will infect rhesus monkeys and produce AIDS, even though HIV-1 will do neither. Groups in France and Sweden have begun to test this possibility and seem confident it will work on the basis of early signs. Finally there is a hint from separate laboratories in the United States and Italy that rabbits can be infected by HIV-1.

In the absence of a simple and reliable animal model for AIDS it is inevitable that pressures will mount for more exploratory trials of candidate AIDS vaccines in at-risk, or HIV-infected, volunteers. Such trials will need careful scrutiny before approval and should not be implemented without fully informed consent as they are not without imaginable risk. But there is little option other than to allow the best of them to proceed. **Peter Newmark**



## Neuroscience

## Stimulus selectivity of single neurons in the temporal lobe

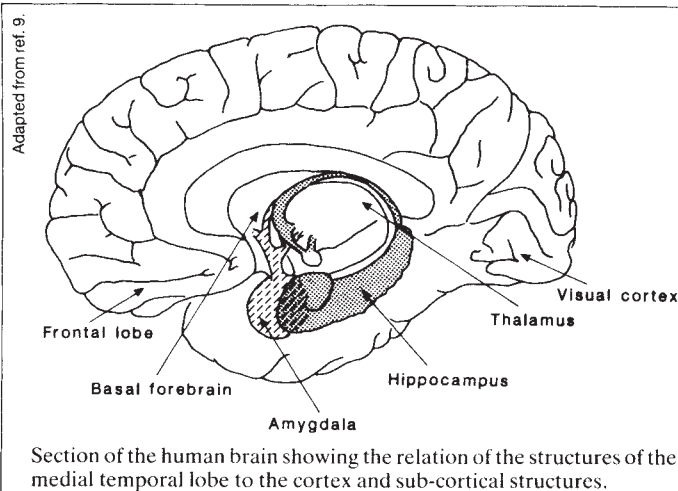
Michael Rugg

THE crucial contribution to memory made by the structures of the medial temporal lobe of the brain — the hippocampus, amygdala and adjacent cortical regions (see figure) — has been investigated by examining the effects on learning and memory of damage to these structures. A complementary approach to elucidating the functions of medial temporal-lobe structures, that of recording from single neurons during behaviour, has been systematically applied only in non-human species. An advance towards bridging this gap has now been made by Heit *et al.*, who on page 773 of this issue<sup>2</sup> describe the activity of single neurons in the medial temporal lobes of epileptic patients in whom intracerebral electrodes have been implanted for clinical reasons. A high proportion of these neurons displays remarkable selectivity for individual members of a repeatedly presented set of 10 words. While one cell shows enhanced firing each time the word 'luck' is presented, for example, another responds preferentially to 'carve'.

A striking feature of these data is the frequency with which word-specific responses were observed; 75 per cent of the neurons from which recordings could reliably be obtained show selectivity for at least one of the 10 words in the list tested. In view of the restricted nature of this list, such a high rate of success in finding word-selective cells implies that each word must have actually evoked activity in many neurons. Together with the fact that some cells are responsive to more than one word, this suggests that words are represented in this neural population by virtue of the pattern of activity they evoke. Such a form of stimulus representation is one of the cornerstones of the 'parallel distributed' models of brain function that are currently under intensive development<sup>3</sup>.

The findings of Heit *et al.* therefore provide support for the view that complex stimuli such as words are subject to some form of population coding in the human brain, but the relevance of these data to understanding the role played by the medial temporal lobe in learning and memory is less clear. Bilateral lesions of this region severely impair recognition memory — the ability to remember whether a particular word (or any other

stimulus) has been encountered in the recent past — but do not interfere with the comprehension and usage of words in everyday language. It therefore seems likely, at least with familiar stimuli such as words, that structures within the medial temporal lobe contribute to memories about the specific episodes in which a stimulus is encountered, rather than about aspects of the stimulus that remain con-



stant across different episodes, such as a word's meaning or pronunciation.

This being so, it is perhaps surprising that Heit *et al.* find that their cells show the same level of firing when the preferred word is shown on the first occasion as when it is repeated, implying that these cells do not encode the information necessary to recognize the re-occurrence of the word. The interpretation of this finding favoured by Heit *et al.* is that the output of the medial temporal lobe to other brain regions does not change as a consequence of stimulus repetition. But this conclusion can be questioned on several grounds.

First, recordings from the hippocampus of macaque monkeys reveal changes in neuronal activity dependent on the context in which a stimulus is presented, or that occur as a consequence of stimulus repetition<sup>4,5</sup>. Thus, in a species closely related to man, there is evidence that some hippocampal neurons do show memory-related changes in their firing rates.

Second, before concluding that the human medial temporal lobe does not change its output when stimulus repetition occurs, it is necessary to demonstrate that the cells sampled actually include ones that reflect its efferent activity. This has yet to be achieved.

Third, Heit *et al.* report that locally

recorded averaged evoked potentials do change with stimulus repetition, indicating that some type of neuronal activity within the medial temporal lobe discriminates repeated from unrepeated stimuli. This activity could be related to efferent information, unavailable to the cells from which unit recordings were obtained by Heit *et al.*, which signal the occurrence of stimulus repetition to other brain regions.

Another way of viewing the data of Heit *et al.* is to compare the cells they describe with the 'place' cells in the hippocampus of the rat. Place cells are so named because of their sensitivity to the animal's location in its environment. They fire selectively each time the same spatial location is entered, and may be part of a parallel distributed system representing the current spatial environment<sup>6</sup>. In 1978, O'Keefe and Nadel<sup>7</sup> suggested that the hippocampus of higher species represents non-spatial information in an analogous fashion, forming so-called cognitive maps of, for example, the semantic attributes of words. The data of Heit *et al.* could provide evidence for such non-spatial representations in the human hippocampus and adjacent structures. For the reasons noted above, such representations are unlikely to be necessary for the retrieval of attributes of a stimulus such as its meaning, but may be used in forming associations between a stimulus and the context in which it is presented.

A final interesting feature of the findings of Heit *et al.* concerns the diversity of the structures in which they find 'word-specific' neurons, including the hippocampus and the amygdala, two structures which make separate contributions to memory, as shown by lesion studies in primates<sup>8</sup>. The existence of stimulus-specific neurons with the same properties in these two structures suggests that, although they perform different functions, the hippocampus and amygdala use a common means of representing a stimulus undergoing processing. Whether other brain regions possess neuronal populations that represent complex stimuli in a similar way is an interesting question for the future. □

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## Planetary geology

## Odd tectonics of a rebuilt moon

William B. McKinnon

MIRANDA, the small icy satellite of Uranus, is perhaps the most bizarre world in the Solar System. Its appearance is unlike any other, and it resembles nothing so much as the joined fragments of several different and unrelated moons. Naturally, many explanations have been advanced, but only now has a leading hypothesis been put to the test, by Janes and Melosh<sup>1</sup>. Remarkably, their sinker tectonics model agrees with many of the observations. How this model came to be is an interesting story in itself.

After the Voyager encounters with Saturn, Eugene Shoemaker put forward an extraordinary proposition: each of the smaller inner satellites of Saturn had been repeatedly disrupted early in its history by hypervelocity impacts of cometary nuclei<sup>2</sup>. This destruction is only temporary, however, because Saturn's gravity confines the resulting fragments in close similar orbits. The tormented satellite reacretes, its geological clock reset. Of course, this was an inference based on the presumed number of large comets, and geological evidence of any sort for the model was lacking<sup>3</sup>. Why did the largest reaccreting fragments not lead to a juxtaposition of different terrain types? If a satellite originally undergoes any substantial differentiation, should not pieces of the old carbonaceous silicate-rich core be thrown together with the icier mantle? Surely there should at least be mysterious albedo boundaries. The answers given,

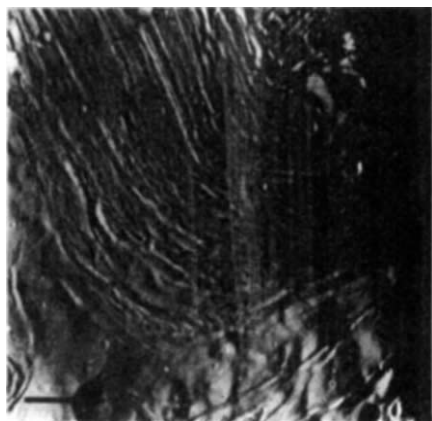


Fig. 1 Voyager image of south-east corner of Elsinore Corona. Linear to arcuate bands of ridges and troughs surround a poorly seen central zone at top right. Many ridges show a medial valley. The short arrows outline a section of cratered terrain apparently by-passed by the ridge-forming process. Although the ridge is possibly a compression feature, it could also be volcanic. The long arrow indicates a small outlying ridge segment. Scale bar, 25 km. (Frame FDS 26846.29, courtesy of P. M. Schenk.)

for example that water-ice frost would conceal such details, were less than satisfactory.

The startling close-up images of Miranda, when first received, answered all these objections. Amid rolling cratered terrain were found one trapezoidal and two ovalar (in plan) younger regions<sup>4</sup>, now termed coronae (Fig. 1). These are characterized by an inner core of intersecting ridges and troughs surrounded by a series of subparallel and concentric bands. These regions generally contain the darkest (and brightest) surface materials as well. Shoemaker promptly proposed that each of the 250-km diameter regions (a size comparable to the satellite's radius) was the tectonic and volcanic result of the late reaccretion of a relatively dense and massive fragment that sank slowly through Miranda's mantle towards its centre. This is tenable because such collisions are quite gentle, occurring at velocities under  $0.2 \text{ km s}^{-1}$ , so although the fragment could disaggregate, it would not be dispersed as in a hypervelocity impact.

It is another matter whether the surface expressions of such sinkers are actually observed. Laurence Soderblom subsequently proposed that Miranda is not necessarily a world on its *n*th reincarnation, but is rather one caught in the throes of its first differentiation<sup>5</sup>. In this picture, silicates sink under gravity, but are part of an overall convection pattern. What allows the rock fraction to separate out is the melting of low-temperature ices such as ammonia hydrate, so that it is buoyant plumes of hot ice-rich material — or risers — that create the coronae.

Janes and Melosh<sup>1</sup> solve an analytical version of the sinker model. Miranda is idealized as a sphere with a rigid core surrounded by an icy mantle of constant viscosity, which is in turn surrounded by an elastic lithosphere. A sinker mass is placed in the mantle and the flow field it sets up is calculated (Fig. 2). This flow creates tractions at the base of the lithosphere, which generate stresses in the lithosphere. At this point a tedious exercise in Legendre polynomials becomes interesting, for it is in the geological interpretation of the stress fields that the model sinks or swims. For a nominal model of a sinker placed 20 km below a thin 5-km lithosphere (and above a core of half the satellite radius), reasonably strong surface compressive stresses are generated within about  $15^\circ$  of the point directly above the sinker. Beyond this distance, stresses distributed throughout the thickness of the shell are the most important, and the

latitudinal stress remains compressive and significant out to about  $45^\circ$ . The parallel ridges and troughs of the coronae, according to Janes and Melosh, originate as compressional folds. The compressional stresses close to the sinker location have no preferred orientation, implying that various fold orientations arise, and the latitudinal compressive stresses farther out could lead to folds preferentially orientated concentrically around the sinker (Fig. 2, top). This is consistent with the general structure of the coronae, with

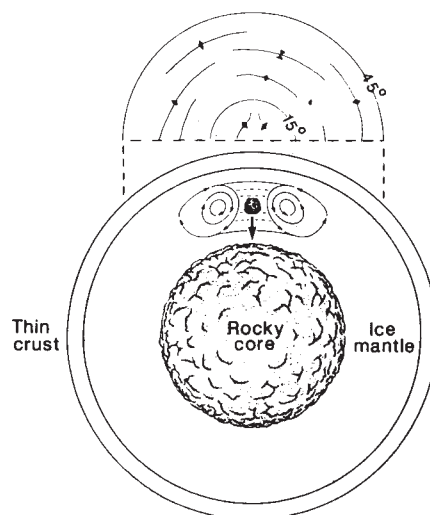


Fig. 2 Schematic version of sinker model geometry (bottom), with actual flow lines for the case described in the text. The expected surface tectonic expression is shown in the cutaway drawing above. (Adapted from ref. 1.)

their inner zones and banded peripheries. The real question then is whether the ridges and troughs are actually folds.

The authors point out that the features of the coronae are generally open to interpretation. Perhaps the ridges and troughs are actually horsts and graben formed by extension. The sinker model can be transformed into the riser model by simply switching the sign of the density contrast at depth. The stresses induced in the lithosphere are then just opposite in sign to those of the sinker, and extensional features with the same orientations as before are predicted.

There remain unresolved puzzles, though. One concerns the role of extensional longitudinal stresses, predicted in the sinker model to be significant beyond  $15^\circ$  from the sinker location. Will concentric compressional folds form in this situation? Another concerns the ubiquitous (and spectacular) normal faulting and graben of the cratered terrain<sup>6,7</sup>, strongly indicative of an important global role for extensional tectonics at some time. A third concerns how the observed resurfacing of the coronae fits into the general scheme of compressive sinker tectonics. The coronae have definitely been resurfaced. Their albedos are different and

they have far fewer superposed impact craters than the cratered terrain<sup>7</sup>.

Janes and Melosh suggest that the accretional collision could have melted some portion of Miranda's surface, which flooded over the sinker's site. Such melting would have to involve volatile ices (or perhaps the mobilization of methane clathrate<sup>8</sup>), as the low velocities noted above are not sufficient to melt water ice<sup>9</sup>. Perhaps a more plausible suggestion is that enhanced radiogenic heating by the silicate-rich sinker causes melting or mobilization of volatile ices at depth, which can then erupt to the surface. Melosh also suggests viscous relaxation could be involved, although there is no specific evidence for this (there are no partially relaxed craters, for example).

I suspect these lines of argument will turn out to be crucial to the acceptance or rejection of the sinker hypothesis. After all, resurfacing by volcanism is a natural component of the riser model. I would even go so far as to argue that the ridges in

Elsinore Corona (Fig. 1) are not tectonic at all, but are primary volcanic constructs, arcuate-to-linear extrusions of highly viscous ice lava complete with medial valleys. In addition, the discussion above has not touched on why sinkers or risers should operate in such a presumably cold and rigid body. Chaotic orbital dynamics and tidal heating are probably the key (see ref. 10), but that is another story. □

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## Malaria vaccines

# The shape of things to come

F. E. G. Cox

RADICALLY new approaches to the development of a vaccine against malaria are likely to emerge as a result of two new discoveries. One suggests that aldolase, a key enzyme in glycolysis, produced during the maturation of the blood stages of the parasite, might be a possible target for immunological attack<sup>1</sup>, and the other points to the possibility of delivering an oral vaccine against stages in the liver in transformed *Salmonella*<sup>2</sup>.

The life-cycle of the human malaria parasite *Plasmodium falciparum* is complex and involves an infective stage, the sporozoite; dividing stages in the liver and in the blood; and non-dividing sexual stages. Malaria parasites can reinfect humans repeatedly. With the increased resistance of the parasite to drugs like chloroquine and the failure of mosquito-control methods, the need for a vaccine against this devastating disease is critical.

Most of the research aimed towards developing malaria vaccines has concentrated on the surface antigens of the non-dividing infective and sexual forms of the parasite and on the dividing forms in the blood. Several candidate antigens, all characterized by short repeated sequences of amino acids that provide an easy target for the immune response have been identified<sup>3</sup> and some have now been used in encouraging but not totally successful vaccination trials<sup>4,5</sup>. One problem with these antigens is that they could be diverting the immune system away from more vulnerable targets<sup>6</sup> such as enzymes.

Enzymes have frequently been considered as targets for chemotherapy but rarely as targets for immunological attack. But a group at the Hoffman la Roche Laboratories in Basel and the University Cantonal Hospital in Geneva, led by Ulrich Certa<sup>1</sup>, has now identified malarial aldolase as one such target. Using standard gene-cloning techniques, the researchers have implicated a previously identified polypeptide of relative molecular mass 41,000, termed p41, associated with parasites maturing in the red blood cells. The p41 polypeptide has been shown to be partially protective against malaria in monkeys<sup>8</sup>, and is 60 per cent homologous to mammalian aldolase. The importance of this enzyme is that the blood stages of the malaria parasite lack a citric-acid cycle and so use vast amounts of glucose — glucose uptake by infected cells is 10–100 times that of uninfected cells<sup>9</sup>. Aldolase catalyses the splitting of fructose-1,6-bisphosphate into glyceraldehyde 3-phosphate and dihydroacetone phosphate, thereby acting as a limiting step in glycolysis. The inhibition of aldolase activity would therefore totally inhibit the maturation of the parasite and subsequent invasion of fresh red blood cells.

There are several advantages of using aldolase as a target: it does not possess the repetitive sequences, characteristic of other malaria antigens, that might divert the immune response, and it does not exhibit allelic polymorphism thought to confer species or strain specificity on other

candidate antigens. Thus there is a real possibility that aldolase could be a basis for a vaccine against many, if not all, types of malaria. On the other hand, the partial homology with human aldolase and the consequent possibility of inducing auto-immunity is a matter of some concern.

So far, all experimental vaccines against malaria have had to be injected, usually with an adjuvant. This is not only labour intensive and expensive but may even be dangerous in countries where the AIDS virus coexists with the multiple use of hypodermic needles. Jerald Sadoff and his colleagues at the Walter Reed Institute of Research in Washington, in collaboration with the Smith, Kline and French Laboratories, show<sup>2</sup> that it is possible to develop an oral vaccine. They inserted the gene for the dominant antigen of the sporozoite, the infective stage injected by a mosquito, into attenuated *Salmonella typhimurium* which, when given orally to mice, inhibits the development of the liver stages. This occurs in the absence of antibody, confirming the growing opinion that immunity to these stages is cell-mediated. As genes for the human malaria parasite *P. falciparum* can be inserted into the human form of *Salmonella*, and as humans can respond to similarly transformed bacteria, there is no reason why a vaccine should not be developed. Again the advantages are that such a vaccine is unlikely to be species or strain specific.

What is the future for malaria vaccines? The conventional view of a cocktail of surface antigens from various stages of the parasite's life cycle is beginning to be replaced by the concept of another kind of cocktail. This would probably consist of antigens in transformed bacteria (or viruses) that would induce a cell-mediated response directed against the liver stages, together with enzyme-based antigens inducing antibody-mediated responses against target enzymes associated with the blood stages. In due course, antigens against the sexual forms could be added. It is also possible that antibodies against enzymes such as aldolase could be used to target drug delivery to predetermined components of the parasite's metabolism. The highly conserved nature of parasite enzymes renders them less likely to undergo selective changes resulting in drug resistance than more conventional chemotherapeutic targets. □

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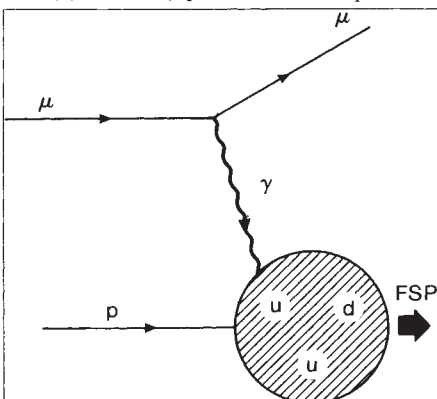


## Proton structure

## Quark spins add up to nothing

C. T. Sachrajda

AMONG the fundamental properties of any elementary particle is its intrinsic angular momentum or 'spin'. The European Muon Collaboration (EMC) has just obtained new results on the spin content of the proton which are somewhat unexpected and have significant theoretical implications (Ashman, J. *et al. Phys. Lett. B* **206**, 364–370; 1988). Among the more surprising conclusions which can be drawn from their measurements are: first, that only a small fraction,  $1 \pm 12$  (statistical)  $\pm 24$  (systematic) per cent of the proton's



**Fig. 1** Schematic representation of the collision of the muon-proton ( $\mu$ - $p$ ) scattering process. The scattering takes place by means of the exchange of a photon ( $\gamma$ ). A shower of final state particles (FSP) is produced from the proton. The target proton consists of three valence quarks (up, u and down, d) and a quark-gluon sea (shaded).

spin is carried by quarks; and second, that the strange quarks and antiquarks in the proton are 'polarized', tending to have their spins aligned in the opposite direction to that of spin of the proton.

The proton is a composite particle, composed of three valence quarks, together with a sea of quark-antiquark pairs and gluons (the mediators of the strong nuclear force). In principle, quantum chromodynamics (QCD) — the theory of quarks, gluons and their interactions — should enable us to understand how the orbital angular momenta and spins of the constituents combine to make up the spin of the proton. In practice, however, such calculations are only slowly becoming possible in QCD, and much of our intuition comes from the non-relativistic quark model. In this model, the proton is viewed as an atom consisting of three slowly moving quarks with total orbital angular momentum equal to zero, so that the proton's spin is entirely the sum of the spins of the quarks. The EMC results seem to indicate that this simple picture is wrong.

In the new experiments, the EMC scatters muons (particles with the properties of electrons but with a mass about 200 times larger) on protons. The scattering takes place by means of the exchange of a photon (Fig. 1). The events of interest are those in which there is a large transfer of energy and momentum from the muon to the proton. The exchanged photon has a very small wavelength (typically about  $0.1 \text{ fm} = 10^{-16} \text{ m}$  or less) and hence it probes well inside a proton (which has a radius of about  $1 \text{ fm}$ ). Such experiments provide fundamental information about the structure of the proton.

The new experiments are performed with polarized muons and protons, so that the orientation of the particle spins is known. The EMC measures the structure function  $g_1^p$  which is given by

$$g_1^p(x) = \frac{1}{2} \left[ \frac{4}{9} \Delta u(x) + \frac{1}{9} \Delta d(x) + \frac{1}{9} \Delta s(x) \right]$$

where for each 'flavour' of quark ( $q$ ), up ( $u$ ), down ( $d$ ) or strange ( $s$ )

$$\Delta q(x) = q^+(x) + \bar{q}^-(x) - (q^-(x) + \bar{q}^+(x))$$

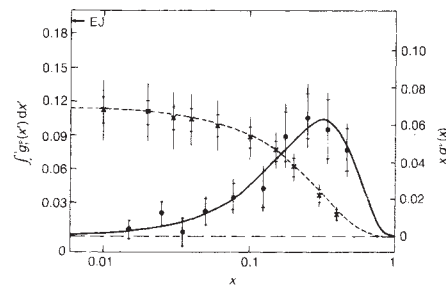
The arrow pointing up or down indicates that the spin of the quark  $q$  or antiquark  $\bar{q}$  is aligned parallel or antiparallel to the spin of the proton. Thus  $q^+(x)$  is the probability distribution of finding a quark  $q$  with its spin aligned with that of the proton and with a fraction  $x$  of the proton's momentum (in a frame of reference in which the proton is highly relativistic). The factors  $4/9$  and  $1/9$  are the squares of the charges of the up quark (whose charge is equal to  $2/3$  of that of the proton) and the down and strange quarks (with charges  $-1/3$  of that of the proton). These factors are present because the probability for the absorption of the photon as in Fig. 1 by a quark is proportional to the square of its charge. The EMC measurements of  $x \times g_1^p(x)$  are plotted as circles in Fig. 2.

A quantity of particular theoretical interest is the integral  $I_p$  of  $g_1^p(x)$  over all  $x$ . EMC obtains the value  $0.114 \pm 0.012 \pm 0.026$ , which conflicts with the Ellis-Jaffe sum rule (Ellis, J. & Jaffe, R. *Phys. Rev. D* **9**, 1444; 1974) that predicts  $0.189 \pm 0.005$ . Because the only dynamical assumption used in the prediction is that the strange sea is unpolarized, this assumption seems to be wrong. Moreover,  $I_p$  is the missing link needed to determine how much of the proton's spin is carried by each of the three quark flavours. From the measured value, EMC concludes that the up quarks and antiquarks combine to have a spin equal to  $74 \pm 9$  per cent of the proton's spin, and that the down and strange quarks tend to have

their spins aligned in the opposite direction to that of the proton, the down quarks carrying  $-51 \pm 9$  per cent and the strange quarks  $-23 \pm 9$  per cent of the proton's spin. The total spin carried by all three quarks is then about 1 per cent (with a relatively large error) as reported in the opening paragraph.

F.E. Close and R.G. Roberts (*Phys. Rev. Lett.* **60**, 1471–1474; 1988) challenge the validity of these results, claiming that EMC underestimates the contribution to  $I_p$  from the region in which  $x$  is very small for which there are no data. The measured values of the integral of  $g_1^p(x')$  from  $x' = x$  to  $x' = 1$  are plotted as crosses in Fig. 2. EMC use the simplest theoretical model to extrapolate this curve to  $x=0$  to evaluate  $I_p$  and find a very small contribution to the integral from the small- $x$  region. Close and Roberts show that, by using a different theoretical model, it is possible to obtain a larger contribution from this small- $x$  region, (perhaps accounting for up to half of the quark contribution to the proton's spin). In this case, however, the quarks at small values of  $x$  would have to be significantly polarized, a result which itself is unexpected.

The EMC results are unexpected, but can they be understood *post-facto*? S.J. Brodsky, J. Ellis and M. Karliner (preprint) show that the Skyrme model does give results broadly in agreement with the EMC measurements. In particular they find that the quarks carry only a small fraction of the proton's spin. This model (in which the proton is an extended stable configuration of the pion field), although approximate, contains many of the features of the full QCD theory. The results are encouraging and raise the hope that it will soon be possible to get an intuitive understanding of the EMC results. Eventually, perhaps within the next five years, it is hoped that by using large computers it



**Fig. 2** Data from the EMC experiment on the spin structure of the proton. Abscissa, the fraction  $x$  of the proton momentum carried by the scattering quark. Right-hand ordinate ( $\bullet$ ),  $xg_1^p(x)$  where  $g_1^p(x)$  is the structure function of the proton. Left-hand ordinate ( $\times$ ), the integral from 1 down to  $x$  of the structure function. According to the EMC this value asymptotically approaches  $0.114 \pm 0.012 \pm 0.026$ , which is at variance with the Ellis-Jaffe sum-rule value (EJ) of  $0.189 \pm 0.005$ . The solid and broken curves are derived from an analytic fit of the spin data. (From Ashman, J. *et al.*)

will be possible to compute quantities such as  $I_p$  from first principles in QCD.

One interesting implication of these results concerns attempts to detect cold dark matter (J. Ellis, R.A. Flores and S. Ritz, CERN preprint), essential to some theories of cosmology. Among the candidates for cold dark matter are the massive, weakly-interacting, neutral particles predicted in supersymmetric theories, in particular the photino. It is hoped to detect these particles either directly in the laboratory, by observing their scattering off matter, or indirectly by observing the neutrinos which emerge when these particles annihilate in the Sun. Predictions of the detection rates for these particles will have to be revised because of the EMC results. The rate for photino-proton scattering, for example, is proportional to

the square of  $I_p$ , and hence is reduced by a factor of about 3.

The EMC measurements are the latest in a series of unexpected results in spin physics. They are perhaps simpler than the previous ones to interpret, in that they involve the wavefunction of only one proton. The results all indicate that our intuitive picture of the origin of the proton's spin, based on the non-relativistic quark model, is wrong. This leaves the following fundamental question: if the quarks carry very little of the proton's spin, then what is the origin of the proton's spin? Does it arise from the orbital angular momenta of the proton's constituents or from the spins of the gluons? □

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## Vertebrate palaeontology

# In search of earliest tetrapods

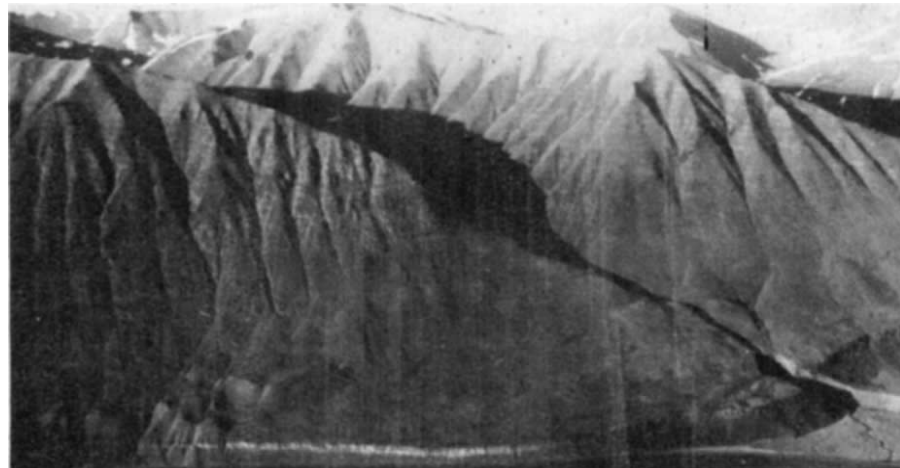
Alec L. Panchen

In that period of the Carboniferous which corresponds to the British Coal Measures, there was enormous radiation of land vertebrates, tetrapods, which include the earliest recorded reptiles and even the earliest mammal-like reptiles. But in the period of the Carboniferous before the Coal Measures, roughly corresponding to the Mississippian of North America, and even more in the preceding Devonian period, tetrapod fossils come from very few localities, are rare as specimens and frequently poorly preserved. Two new papers add significantly to our knowledge of the anatomy and distribution of these earliest land vertebrates. On page 768 of this issue<sup>1</sup>, Bolt and colleagues describe a new vertebrate locality from Iowa which contains many well-preserved fish and tetrapod fossils. This is the earliest productive tetrapod site in continental North America. In the second paper<sup>2</sup>, Clack adds to our knowledge of one of the two principal tetrapod genera from the Devonian of Greenland, preliminary to her successful visit to the localities.

The new site described by Bolt *et al.* is important for three reasons — its early date, its geological location and the nature of its fossils. Before its discovery, tetrapods of Mississippian age were known from three principal areas: the environs of Edinburgh; Nova Scotia; and northern West Virginia and the adjoining part of Pennsylvania<sup>3</sup>. There are Coal Measures sites in Illinois, but the Iowa find is the first Mississippian site to be reported from mid-continental North America and the furthest west yet discovered. It is definitely Lower Carboniferous in date, being of middle Viséan age, and thus corresponds to a horizon in the Edinburgh Oil

Shale Group from which the earliest recorded Scottish tetrapods came.

One of the tetrapod fossils from Iowa is a member of the family Colosteidae, whose members are also known from the Edinburgh Oil Shales and above, from the later Mississippian of West Virginia, and



Uphill task — Stensiö Bjerg, Greenland, where the collections of *Acanthostega* were made in 1987.

from the Coal Measures of Ohio<sup>4</sup>. The other principal tetrapod find represents a new taxon of very considerable interest. Bolt *et al.* refer to it as a 'proto-anthraco-saur' and, rightly in my opinion, believe it to be closely related to, but not a member of, the Anthracosauria. The anthracosaurs are a group of primitive tetrapods, ranging in time from the Oil Shales to the early Permian and are thought by some to be closely related to amniotes (reptiles, birds and mammals). The proto-anthraco-saur has some of the diagnostic features of the group, but it is primitive in the configuration of the bones of the skull table and in having possibly the most primitive type

of tetrapod vertebra. It joins the controversial *Crassigyrinus* from the Scottish localities in showing that anthracosaur characters can co-exist in one animal with extremely primitive features<sup>5</sup>.

Clack<sup>2</sup> gives an account of much earlier tetrapods. The material was collected in 1970 by Peter Friend and colleagues from Cambridge while they were exploring the stratigraphic geology of the area in which lie the classic East Greenland Late Devonian tetrapod sites (see figure). The commonest tetrapod from these sites is *Ichthyostega*, but Clack's new account is of three closely associated skulls of *Acanthostega*, known previously from only two incomplete skull roofs. The new specimens yield data on the braincase (typically tetrapod), lower jaw and shoulder girdle. Clack has just made a successful visit to East Greenland and collected about three-quarters of a tonne of Devonian tetrapod specimens.

The expedition on which Clack collected her data<sup>6</sup> was supported by the Greenland Geological Survey. It was an Anglo-Danish initiative led by Svend Bendix-Almgreen (Copenhagen), accompanied by Birger Jørgensen (Copenhagen), Jenny and Rob Clack and Per Ahlberg (Cambridge). The specimens collected seem to be mostly *Acanthostega*, but include *Ichthyostega*, which will supplement data from specimens already in Stockholm<sup>7</sup>. The new material will be

described in Cambridge and important specimens then returned to Copenhagen. The material from East Greenland is the only substantial collection of these earliest land vertebrates. □

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## Supernova 1987A

## Casting light on the ejecta

Robert D. Gehrz

SUPERNOVA 1987A, 165,000 light years away in the Large Magellanic Cloud, gives astronomers an unprecedented opportunity to test theories of the evolution and core collapse of massive stars. As the ejecta from SN1987A expand and thin, infrared and visible signals should become increasingly useful for identifying the elements created in both the progenitor and explosion, and the physical state of matter in the cloud. Already it is clear

decline, and as the ejecta become optically thin,  $\gamma$ -ray lines from  $^{56}\text{Co}$  decay should appear<sup>4,6</sup>. Recent observations of 847- and 1,238-keV  $\gamma$ -lines<sup>7,8</sup> show that radioactivity is at least partially responsible for powering the ejecta.

Michel *et al.* propose<sup>9</sup> that the core is a pulsar (a rotating neutron star) and that this powers the ejecta. The decline of visible light is caused by the loss of energy by  $\gamma$ -ray emission as the ejecta become

transparent at short wavelengths. In this case, the leaking  $\gamma$ -rays will have a continuum energy distribution. Some continuum  $\gamma$ -rays are becoming visible now<sup>8</sup> but it is not yet clear whether these give a view of the central source.

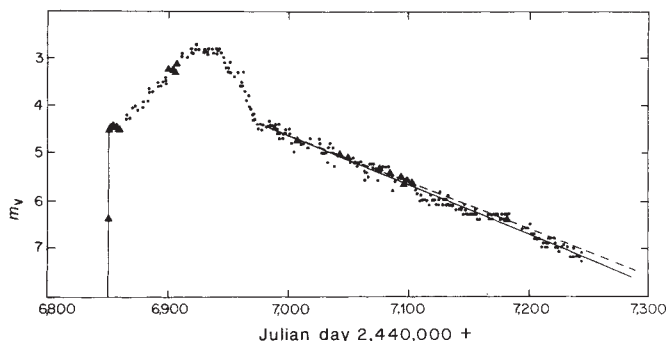
A stable neutron star could become evident in other ways. When the ejecta become transparent in several years, pulsed radiation could be

thick, absorbing all the photons except  $\gamma$ -rays, it will be a calorimeter of the electromagnetic luminosity of the central engine.

Estimates of the time for dust to form depend upon the expansion velocity of the ejecta containing the dust condensates and the development of the luminosity of the central engine. These parameters determine when the ejecta reach the condensation point. The best guesses, based on the low outflow velocities recently reported for the heavy element rich ejecta, range from during the coming year to 10 years from now. Measurements of the linewidths of infrared lines from atomic iron, indicative of the degree of thermal motion, show that the temperature of the ejecta is still 3,000 K (ref. 2). Dust cannot form until the temperature is about 1,000 K, and judging from the rate of expansion, this could take up to 10 years if the luminosity of the central engine remains constant.

The  $\gamma$ -rays from the  $^{56}\text{Co}$  radioactivity could inhibit dust formation. A recent infrared spectrum<sup>10</sup> shows an emission line possibly due to cobalt. Because the processed ejecta are not yet optically thin, this observation tends to confirm the suspicion that some  $^{56}\text{Co}$  has been mixed into the outer ejecta. If so, the ejecta may be glowing throughout, and dust could not efficiently block visible light unless it were concentrated in an outer envelope. Neither the visible light curve (Fig. 1) nor the infrared fluxes, however, suggest that dust is forming yet.

Light 'echoes' have been observed (Fig. 2). These are attributable to direct heating of pre-existing dust<sup>15</sup> produced in the wind of the supergiant precursor and to reflections of the explosion off interstellar clouds<sup>16,17</sup>. Because light radiates from the supernova at the speed of light, it can reflect off materials far beyond the dust



**Fig. 1** The visible-light curve of SN1987A as reconstructed from visual estimates (closed circles) and photometry (arrows) reported in IAU circulars. The best fit (solid line) to all the data suggests that the visible light is now decaying with a half life of 72.5 days, very close to the 78-day half life (broken line) for the radioactive decay of  $^{56}\text{Co}$  to  $^{56}\text{Fe}$ .

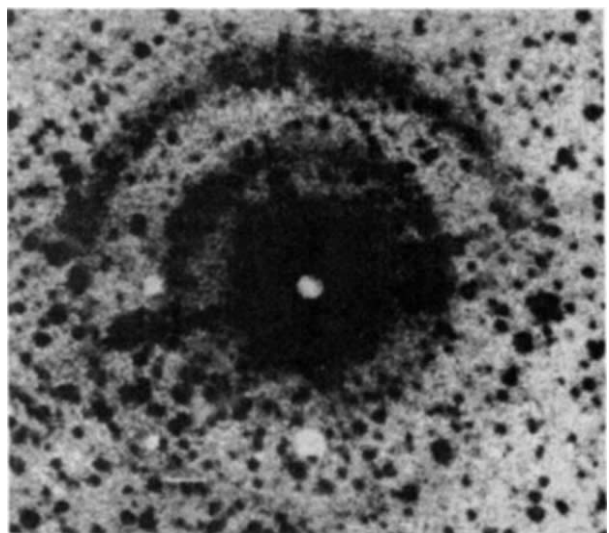
from infrared detections that strong mixing in the ejecta has brought up material from close to the core<sup>1</sup> and that the inner zones of the cloud are still so hot<sup>2</sup> (at about 3,000 K) that condensation to form dust may not occur for 10 years.

An important issue is the fate of the collapsed core of SN1987A. A type II supernova explosion should eject 95 per cent of the stellar mass at velocities of 2,000–20,000 kilometres per second, leaving behind either a black hole or a rapidly rotating neutron star, depending on the mass of the collapsed core. The neutrino bursts observed by the Kamiokande II and IMB detectors are consistent with this model, SN1987A arising from the gravitational collapse of the fuel-exhausted iron-rich core of a 20-solar-mass star to form a stable neutron star, although black-hole formation cannot be excluded<sup>1</sup>.

The recent decline rate of the visible light from SN1987A (Fig. 1) is close to the 78-day half life of radioactive  $^{56}\text{Co}$  in the  $^{56}\text{Ni} \rightarrow ^{56}\text{Co} \rightarrow ^{56}\text{Fe}$  decay chain<sup>4</sup>. As much as a tenth of a solar mass of  $^{56}\text{Ni}$  could have formed in the explosive nucleosynthesis prompted by the core collapse<sup>3</sup>. It seems remarkable that the visible-light intensity is declining at the rate predicted for radioactivity. If radioactivity is dominating the luminosity then it should be the total (or bolometric) energy output rather than any monochromatic flux that follows this

observed from the relativistic particle beam accelerated by the rotating neutron star<sup>9</sup>. The light decline should halt when the energy absorbed by the ejecta from the central pulsar is greater than the luminosity produced by radioactive decay<sup>4,9</sup>. The onset of transparency will also enable observers to see the rich optical and infrared line spectrum that should reveal the nature of the nucleosynthetic processes during the evolution of the precursor<sup>6</sup>. These lines are now becoming observable<sup>1,2,10–13</sup> in the ejecta.

Astronomers have long suspected that grains can condense in the wind of ageing supergiant stars and in the ejecta of the type II supernova explosions produced by the core collapse of these massive stars. SN1987A is the first supernova in our lifetime bright enough for comprehensive infrared documentation of the process of dust formation. As well as emitting thermal infrared radiation<sup>6</sup>, the dust could substantially block the visible radiation from the central engine<sup>14</sup>. If the dust shell is optically



**Fig. 2** Double light echo from SN1987A, recorded on 13 February 1988 by Michael Rosa using the ESO 3.6-metre telescope. The two rings, at 30 and 50 arc seconds, are reflections of light from SN1987A of the interstellar medium.

(Courtesy of ESO.)

condensed in the expanding ejecta. To distinguish light echoes from light generated by the ejecta, measurements of the angular extent of the emitting regions are needed. Reflections of the flash off interstellar material in the foreground several hundred light years from the supernova can create a 'phantom nebula'. Such nebulae can appear to expand at velocities far exceeding the speed of light and are similar to the 'superluminal expansions' of extragalactic radio sources.

The phantom-nebula effect, which provides an elegant proof of the existence of interstellar clouds, has apparently been confirmed for SN1987A by recent photographic observations<sup>16</sup> (Fig. 2). Spectra<sup>17,18</sup> show that light from newly developed arcs 30 and 50 arc seconds away from SN1987A is identical to light emitted by the supernova shortly after its eruption a year ago.

Thermal light echoes caused by direct heating of, or reflections off, pre-existing material from the progenitor will be only an arc second across at present, whereas thermal emission from dust condensing in the ejecta will occur on spatial scales about 30 times smaller. The presence of pre-existing gaseous material has been detected by optical spectroscopy<sup>19</sup>. A

spectacular visual brightening of the supernova could occur in several years when the blast wave runs into material previously ejected during the precursor's supergiant wind<sup>20</sup>. Such re-explosions could explain several reported re-appearances of historical supernovae. Secondary infrared thermal echoes could also result from this interaction<sup>4</sup>. □

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## Agricultural ecology

# Essential elements from waste

Peter D. Moore

THE disposal of human sewage raises ecological problems which are still far from being solved. Sewage sludge, a potentially toxic waste, must be removed from the centres of population where it is produced, rendered harmless and disposed of, often by incineration or by dumping as a land fill. But sewage is also a rich source of many of the elements required for crop production, like nitrogen and phosphorus, which are acquired only at great energetic and economic expense. Its organic content could provide the soil conditioner currently supplied by peat and thus contribute to the conservation of the world's declining wetlands. But there is a drawback. Other toxic components of sewage, particularly heavy metals, present a barrier to its widespread use. Recent work in arable agricultural systems in the United States<sup>1</sup>, and in grasslands in Britain<sup>2</sup>, show that the problems are not, however, insuperable: mycorrhizal fungi could provide a way forward in this important area of agricultural and ecological research<sup>3</sup>.

Day and Thompson<sup>1</sup> conducted experiments in Arizona to test the efficacy of dried sewage sludge as a replacement for conventional fertilizers in the cultivation of wheat. Over three years they supplied

different varieties of wheat crops with the recommended fertilizer levels for Arizona soils (mainly nitrogen and phosphorus, as potassium is considered to be adequate in these soils). At the same time, they applied dried sewage with nitrogen levels equivalent to those in the recommended fertilizers, and they also applied a nitrogen-phosphorus-potassium (NPK) treatment to match the sewage sludge. They find no difference in yield between wheat varieties, but all cultivars produce plants with higher yield in the sludge treatment or with the equivalent levels of NPK. So the sludge is an effective alternative to, and even an improvement on, conventional fertilizer treatments. The heavy-metal content of the grain is significantly greater in the sewage treatment for zinc, lead and nickel, but no significant differences were recorded for cadmium or copper. The levels recorded (45.3, 4.5 and 22.4 mg kg<sup>-1</sup> for zinc, lead and nickel, respectively) are regarded by the authors as "not excessive". But the grain produced

## Erratum

In the article "Action at a distance" by Neville Symonds (*Nature* **333**, 18–19; 1988), Fig. 1 should have been credited to M.G. Surette, S.J. Buch and G. Chaconas (*Cell* **49**, 253–262; 1987). □

is intended for livestock feed and one needs to know more about the long-term accumulation effects in the food chain.

Heavy-metal accumulation can take place not only in food chains, but also in the treated soils, and long-term monitoring of soil levels is clearly needed both in arable and pastoral trials involving sewage treatment. Davis and co-workers<sup>2</sup> are concerned with pasture soils in Britain, where about 20 per cent of the sludge used in agriculture is applied as a dressing to permanent pasture. In a four-year experiment on both calcareous and neutral soils, they find that, on average, 87 per cent of the added heavy metal component becomes concentrated in the upper 5 cm of the soil. In the case of zinc, over the four-year period 94 kg ha<sup>-1</sup> were added in the sludge, which results in an increase of 83.5 mg kg<sup>-1</sup> in the concentration of zinc in the top 10 cm of soil. The ryegrass on the site takes up surprisingly little of this load. Less than 2 kg ha<sup>-1</sup> of the added zinc is taken up into the pasture grass (under 2 per cent of the zinc added in sludge).

One possible source of protection of plants from excessive heavy-metal uptake is the fungal component of mycorrhizas<sup>3</sup>. The role of these symbiotic associations in the accumulation of scarce nutrients to the benefit of the host plant has long been recognized, but they may also play a part in the protection of plants from undue loads of potentially toxic elements. Fruiting bodies of some of the fungi involved in mycorrhizas accumulate heavy metals on contaminated soils, and there is some evidence that the fungal mycelium of the heather (*Calluna vulgaris*) mycorrhiza acts as a filter for heavy metals<sup>4</sup>. Colpaert and van Assche propose<sup>3</sup> that there are heavy-metal-tolerant strains of ectomycorrhizal fungi in such instances, and demonstrate the capacity of some fungal strains to grow well even at zinc concentrations of 1 mg g<sup>-1</sup> (considerably higher than the concentrations recorded in the pasture-soils experiment). The culture and use of such strains could improve the soil/plant barrier for heavy metals in commercially important plant species and facilitate the development of sewage-based fertilization systems of agriculture.

One problem remains for the pastoralist. How can the grazing animal be prevented from eating contaminated soil? Perhaps, as Fleming suggests<sup>5</sup>, pasture soils need to be ploughed occasionally to mix metals in the soil and prevent their concentration in the surface layers. □

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## Vision

## Eye development and short sight

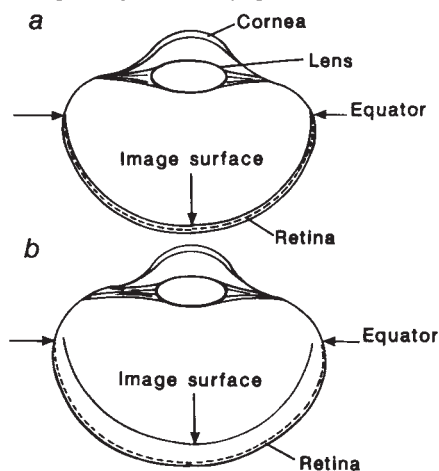
Graham R. Martin

COMPARED with the complex optical structures found in invertebrate compound eyes (see the recent News and Views article by Mike Land<sup>1</sup>), the vertebrate eye appears to be a simple affair, consisting of just two main refractive components, the lens and the cornea. But the quality of the optical image produced in the vertebrate eye is usually far superior to that of invertebrates. This quality can be achieved only by highly accurate coordination of the refractive powers of the lens and cornea and of their positions relative to each other, and to the retina. Three groups, who report their results in recent issues of *Vision Research*<sup>2-5</sup>, are beginning to show how this remarkable coordination of the eye's optical structure is achieved, and how it can break down to produce myopia (short sight).

Not all eyes in the same species have the same optical structure. In their classic comparative study of more than 300 human eyes, Sorsby *et al.*<sup>6</sup> demonstrated that in those eyes judged to be emmetropic (normal eyes which when relaxed bring the image of a distant object to a focus at the retina), the eye's axial length, its corneal and lens refractive powers and its total refractive power, could vary between individuals by more than 20 per cent. These authors concluded that "the emmetropic eye is not the end result of a haphazard combination of variable components, but a co-ordinated organ. The individual emmetropic eye . . . is not accidental." They also showed that in the human eye, refractive errors, such as myopia in which the image is produced in the wrong place with respect to the retina, are usually the result of anomalous components whose values fall outside the range of normal variation. But although the coordinated development of the emmetropic eye seems to depend on normal visual experience, it was not possible to pin down what exactly underlies the apparently uncoordinated development of the myopic eye, or to determine the extent to which the development of an emmetropic eye is controlled by experiential influences as opposed to simple growth. The new research is beginning to elucidate this problem and shows just how sensitive the eye's structure is to anomalous visual input.

The new work of Pickett-Seltner *et al.*<sup>2,3</sup> and of Wallman and Adams<sup>4</sup> exploits simple techniques<sup>7-9</sup> which can cause a growing eye to develop a high degree of myopia. It is even possible to induce myopia in one eye of an animal and not in the other, so an individual can act as its

own control. The most robust technique has been developed for use with chicks and simply involves covering the eye at birth with a translucent goggle. Pickett-Seltner *et al.*<sup>2,3</sup> show that a high degree of myopia is induced by this technique in chicks as young as 14 days old, indicating clearly the importance of patterned visual input to the eye for its coordinated development during this sensitive period. A high degree of myopia can even be



Chick eye with experimentally induced short-sight. In the normal eye (a) the image of a distant object produced by the lens and cornea lies at the surface of the retina. In an experimental eye (b) the equatorial diameter and the axial length of the eye are increased, and the image of a distant object now lies in front of the retina. Hence the eye is described as 'short-sighted' as only the images of objects close to the eye can be focused at the retina.

induced by fitting chick eyes with goggles which restrict the width of the eye's visual field<sup>7</sup>.

But what does the myopia induced by this technique consist of? What parts of the coordinated development of the eye are disrupted? It appears that the whole eye becomes enlarged so that both its axial length and equatorial diameter are increased but the lens remains unaffected. Changes in eye size were evident even within two days of hatching<sup>3,4</sup>, indicating just how sensitive the development of the eye appears to be and showing that this development is dependent on the clarity of vision. Furthermore, the observed size increase in the eye appears to be the result of expansion of the globe resulting from the accumulation of fluid rather than growth of the eyeball tissue itself. The observed phenomena could be explained in part as the result of an inflammatory response of the eye to goggle wear<sup>10</sup>, but the new work suggests

that this is not the case<sup>2</sup>.

Even more intriguing is the finding that this experimentally induced myopia is reversible if the visual restriction is removed during the first 6 weeks of life<sup>4</sup>. If pattern vision is restored during this period, then the eye will become emmetropic with the rate of recovery from myopia directly related to the degree of myopia induced. These results all suggest there is a feedback mechanism which regulates eye growth and which is influenced by the quality of the image present on the retina.

Frank Schaffel and colleagues at Cornell University<sup>5</sup> have now taken the technique one step further. Instead of simply inducing myopia by the use of translucent goggles, they raised chicks with lenses in front of their eyes which slightly defocus the image on the retina. The refractive powers of the lenses used are large enough to blur the image in a normal emmetropic eye but are within the range such that their optical effects can be compensated by the natural accommodation of the chick eye. Schaffel *et al.* show that growing chicks can indeed keep the images on their retinas in focus when wearing the lenses. But when the lenses are removed after between 17 and 26 days, the eye is permanently out of focus — it has developed to take account of the refractive error forced upon it by the lens. As in previous studies, Schaffel *et al.* find that it is eye length that has changed, just sufficient to bring the image and retina into approximate coincidence. Also, as previously, they find the refractive components of the eye are unchanged and the effect occurs independently in each eye.

The dependence of the developing visual areas of the brain on normal visual input has been well established. These new results suggest an intriguing developmental feedback mechanism between the neural retina, where a patterned image is detected, the optical system which produces that image, and growth of the eye. These simple techniques which experimentally induce myopia hold out the promise not only that the mechanism underlying the development of myopia may be understood, but also that the finely coordinated development of the normal eye's optical system might be elucidated. □

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## X-ray pulsars

## Spin-up changes to spin-down

N. E. White

ELSEWHERE in this issue, K. Makishima *et al.* (*Nature* 333, 746–748; 1988) report that the spin-rate of the X-ray pulsar GX1+4, which they have been observing with the X-ray satellite Ginga, is now decreasing whereas previous observations had shown that it was increasing. This fits neither of the two behaviours observed in the other 25 known X-ray pulsars: continuous spin-up lasting for decades or alternate spin-up and spin-down on timescales of hours to months. The unusual behaviour of GX1+4 is probably related to a change in its interaction with material captured from a nearby companion star.

X-ray pulsars are spinning neutron stars capturing material from a binary companion. The strong magnetic field of the neutron star funnels the accreted material down onto its poles where the gravitational energy is released as X-rays. The rotation of this hotspot sweeps the X-rays like the beam from a lighthouse, causing the characteristic pulses observed. Spin-up is expected because the accretion flow contains angular momentum which will be added to that of the neutron star. Spin-down is poorly understood.

X-ray pulsars powered by the capture of wind from a young luminous O- or B-star companion switch between intervals of spin-up and spin-down on timescales of hours to months. Two-dimensional hydrodynamic calculations show that the flow pattern of an OB star's wind to a neutron star is not steady, and can reverse direction. This causes the pulsar to be spun up and spun down in the same direction as the accretion disk. In other cases where the companion fills or nearly fills its Roche lobe (the volume beyond which it sheds gas passively onto the neutron star) pulsars spin up continuously on timescales of up to 50 years, because a steady gas stream feeds the accretion disk.

An X-ray pulsar can also change from spin-up to spin-down because of increased drag on the neutron star's magnetosphere by an accretion disk. This occurs when the co-rotation velocity of material threaded onto the magnetic field lines is comparable to the keplerian (stable-orbital) velocity. If the accretion rate decreases, the radius at which the magnetic field disrupts the accretion disk increases until the keplerian velocity there equals the spin velocity. An appreciable spin-down torque is then exerted even though accretion continues (Ghosh, P. & Lamb, F. K. *Astrophys. J.* 232, 296–316; 1979). At even lower accretion rates, when the co-rotation velocity exceeds the keplerian velocity, accretion onto the neutron star ceases because of

centrifugal forces.

The accretion rate in the GX1+4 system may have decreased just to the critical point where spin-down can occur although accretion continues. The keplerian period in the accretion disk of 110 seconds corresponding to the spin period of the neutron star occurs at a radius of about 40,000 kilometres. A magnetic field of about  $10^{14}$  gauss is required to disrupt the disk at this radius, much more than the field of about  $10^{13}$  gauss usually associated with pulsars.

Makishima *et al.* dislike invoking such a high magnetic field and prefer the model used to explain the episodes of spin-up and spin-down of X-ray pulsars orbiting OB stars. GX1+4 is unique among X-ray pulsars with late-type companions. Its binary companion is an M6III star which could have a substantial wind causing a significant torque on the neutron star. The problem for this model is that the wind of an M6III star is a hundred times slower than that of an OB star. The captured specific angular momentum scales as the fourth power of the velocity and for an M-star companion, a large stable disk rotating in the same sense as the binary will form and is unlikely to reverse direction.

In my view, the model that requires the magnetic field to be about  $10^{14}$  gauss is more likely. This is rather high when compared with the range found from radio pulsars which has a maximum of about  $10^{13}$  gauss, but is not excluded on physical grounds. In fact it has been speculated that the cut off at the high end of the magnetic field distribution of radio pulsars indicates that the elusive radio pulse mechanism only operates over a selective magnetic field range (Radhakrishna, V. *Contemp. Phys.* 23, 207–231; 1982). The X-ray pulsars are pulsed by viewing a rotating beam of X-rays from the magnetic poles, a mechanism not strongly dependent on the strength of the magnetic field.

As noted by Makishima *et al.* in this issue, this observation of spin-down from GX1+4 neatly confirms the view that the rapid spin-up timescale of about 50 years observed in pulsars such as GX1+4 cannot be sustained and that these pulsars must also undergo extended intervals of spin-down. The rate of spin-down of GX1+4 will allow a test of spin-down models and also provide a more soundly based estimate of the number of dormant X-ray pulsars in the Galaxy. □

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## Daedalus

## Psychic chaos

THE evidence for paranormal effects like psychokinesis is very anecdotal and statistical. Most scientists discount it, arguing that psychic forces must be vanishingly small to have evaded detection and measurement all these years, and that in any case a world governed by the laws of physics has no room for them. Daedalus now points out that in 'chaotic' systems the grip of physics is severely weakened.

Chaos is a hot topic these days, and can govern even classically deterministic objects like pendulums. Two different starting conditions of such a chaotic pendulum, even if arbitrarily close together, will rapidly evolve into quite different patterns of swing. So, says Daedalus, the final state of such a pendulum must ultimately be influenced by initial perturbations smaller than its limiting Heisenberg uncertainty. Here is a region, closed to physics even in theory, where extremely tiny psychic effects could be 'amplified' into final dominance.

The largest and most important chaotic system is, of course, the weather. In temperate regions like western Europe, the weather is generated by the continual self-amplification of turbulence between conflicting oceanic winds. On every time- and distance-scale, random fluctuations appear and grow. Current forecasts reach a week ahead; with detailed knowledge of much smaller atmospheric features, two weeks might be feasible; but six weeks ahead the controlling perturbations are below the Heisenberg limit and beyond physics. So, says Daedalus, it may be entirely rational to pray for rain, provided you do it six weeks in advance.

To test this bold speculation, DREADCO's statisticians are studying the weather records of the past century or so. Have events like harvest times and national holidays, the subject of fervent and long-range public wishing, been sunnier than you would expect? If so, Daedalus will try to discover how many people are needed to produce a useful psychic effect. Traditional psychic experiments on single individuals are always infuriatingly inconclusive; a mass experiment should multiply any tiny effects up to convincing reproducibility. So DREADCO's engineers are building a 'chaotic dice' machine in which sub-Heisenberg effects can be amplified up to dominance in a minute or so of tumbling. Daedalus will then use it to play "Wish for the number" games in front of a public meeting or, better, a television audience. If a big audience can indeed control a chaotic dice by mass wishing, psychic research will be revolutionized. Meanwhile, it will soon be time to start dreaming of a white Christmas.

David Jones



## British civil plutonium: production and fate

SIR—In 1985, we reported calculations of the size of the UK civil plutonium stockpile from which we concluded that at least  $2.3 \pm 0.8$  tonnes of plutonium (sufficient for 400 warheads) are unaccounted for<sup>1</sup>. We now confirm the accuracy of our calculations for more than half the total civil inventory by comparison with official figures. Our view that previous government assurances on the fate of civil plutonium are incorrect is supported by recent statements by the British prime minister<sup>2</sup>.

We have extended our calculations of civil plutonium production in the Magnox reactors belonging to the Central Electricity Generating Board (CEGB) and South of Scotland Electricity Board (SSEB)<sup>1</sup> up to 31 March 1987 using government figures for thermal energy generated and spent fuel discharged in fiscal years 1985–86 and 1986–87<sup>3,4</sup>, incorporating the subsequent corrections to the parliamentary record<sup>5,7</sup>. All the reactor-dependent parameters for our calculations were fixed before the government inventory for 31 March 1985 was published and we have not re-optimized our model. The new results reported here are thus predictions.

Official figures for the total civil production of plutonium to date are not available. The figures which the government publishes annually for the UK civil plutonium stockpile<sup>3,8</sup> refer only to a subtotal. The unpublished balance was apparently sent to the United States before 1971 under mutual defence agreements. Because the plutonium was exchanged for highly enriched uranium for the UK defence programme, the government refuses to quantify this balance (for example, see ref. 8). Information on the present civil locations of the plutonium in the United States, however, enables us to estimate that they contain at most 4 tonnes of the plutonium of UK origin<sup>1</sup>.

Despite their incompleteness, the parliamentary answers containing the subtotals of the civil plutonium inventory on 31 March 1986 and 31 March 1987 provide stringent tests for our model. On these dates the differences between our calculated figures for the total plutonium (discharged and in core) and the published official subtotals are 6.82 and 7.14 tonnes respectively, in very good agreement with the average difference of  $6.8 \pm 0.8$  tonnes found for four previous dates<sup>1</sup>. Subtracting the  $0.5 \pm 0.2$  tonnes lost in reprocessing and the 4 tonnes upper limit for the plutonium of UK origin known to be in the United States again leaves  $2.3 \pm 0.8$  tonnes as the balance unaccounted for. We see no need to change this figure in the light of our new results.

Other tests give further confidence in our calculations. The civil plutonium inventory was first published on the same

fiscal year basis as the thermal energy and fuel discharge figures on 31 March 1983. Providing that the unpublished balance of plutonium was removed from the inventory before that date, the recently published figures for the increase in the subtotal should agree with our calculation. This is the case, as our calculated figures for the increases from 31 March 1983 to 31 March 1986 (7.37 tonnes) or 31 March 1987 (9.68 tonnes) lie within 2% of the government figures (7.5 and 9.5 tonnes respectively). Similarly, our calculations for the total plutonium in the cores of the civil Magnox reactors on these dates (9.71 and 10.09 tonnes) also agree to within about 2% with government figures (9.5 and 10.0 tonnes).

In ref. 1 we pointed out that only if we had overestimated plutonium production by 9% could the balance unaccounted for be as small as the amount which could

| Plutonium (kg) discharged in spent fuel during fiscal year 1986–87 |                                             |                                              |
|--------------------------------------------------------------------|---------------------------------------------|----------------------------------------------|
| Station                                                            | Our figures<br>(rounded to<br>nearest 5 kg) | DoE figures<br>(rounded to<br>nearest 50 kg) |
| Bradwell                                                           | 160                                         | 150                                          |
| Berkeley                                                           | 100                                         | 100                                          |
| Hinkley Point A                                                    | 350                                         | 350                                          |
| Trawsfynydd                                                        | 245                                         | 250                                          |
| Dungeness A                                                        | 195                                         | 200                                          |
| Sizewell A                                                         | 200                                         | 200                                          |
| Oldbury                                                            | 285                                         | 250                                          |
| Wylfa                                                              | 170                                         | 150                                          |
| Hunterston                                                         | 220                                         | 200                                          |

have reached Windscale by 31 March 1969 (the cut-off date for the exchanges, according to the CEGB). A parliamentary answer<sup>9</sup> published after our paper suggests that plutonium from Hunterston may have been available for export under the defence agreements up until 1971. Keeping plutonium from different reactors separate would have been contrary to the normal practice of British Nuclear Fuels Limited<sup>10</sup>. Even if the statement about Hunterston plutonium is accurate our calculation would still need to overestimate plutonium production by at least 7% for the industry and government statements about the cut-off dates to be correct.

If we make a 7% reduction in our calculations, in three of the four new tests described above the reduced figure lies outside the limits on the official figures. (Government figures are rounded to the nearest half tonne so the actual quantities must lie within  $\pm 0.5$  tonnes of the official figure in the case of the subtotal increase and  $\pm 0.25$  tonnes in the case of plutonium in core.) The possibility that our calculations are a 7% overestimate is therefore definitely excluded.

In response to a recommendation of the

Sizewell Inquiry inspector<sup>11</sup>, the Department of Energy (DoE) has begun to publish (see (2) and (3) below)<sup>12</sup> reactor-by-reactor figures for plutonium discharge, starting with the fiscal year ending 31 March 1987. Comparison of published and our calculated figures (see table) shows that the correlation between the two sets of figures is very good indeed, with only the calculated figure for one out of nine stations lying outside the range of the DoE figures (which are rounded to the nearest 50 kg).

The comparisons we have made show that the predictions of our model, based on parameters fixed in 1985, agree to within about 2% in all cases with recent government figures. We must now consider if our model could be an overestimate for earlier years. The first reason we think this unlikely is that fuel discharged in the early years of the Magnox programme had a burnup distribution which is similar to that of the fuel currently in the reactor cores. The excellent agreement between our predictions and the official figures for plutonium in core gives confidence that the plutonium production functions are applicable over such a range of burnups.

Second, we made two indirect tests of our calculations for the years before 31 March 1983 in ref. 1. We compared our plutonium production figures for the six fiscal years 1977–83 with the CEGB figures for plutonium dispatched in 1978–84 to Sellafield (formerly called Windscale) from the stations with cooling ponds. Our calculations were 3.6% under the CEGB numbers. We also found that the amount of plutonium which could have been reprocessed before the apparent cut-off date for the exchanges was consistent with the CEGB figure of approximately 4 tonnes<sup>13</sup>.

When we sum, without double-counting, the quantities of plutonium in all the above comparisons we find we have tested our calculations of 60% of the current civil inventory against government and industry figures and found them to be correct within the specified errors. If our overall calculation of the plutonium total is to be an average 7% overestimate, then it must be a very much greater overestimate for the remaining 40% which we have been unable to check. The bulk of this remaining 40% was produced in the period 1970–77. The CEGB claims<sup>14</sup> that information on plutonium production before 1977 has been “erased from the computer memory and hence is no longer readily available”.

The CEGB's non-quantitative response to our work<sup>15</sup> stated that we had not justified our claim to 2% accuracy. We have pointed out<sup>16</sup> that they ignored a number of tests described in our original publication. Furthermore, the agreement demon-

strated here between our predictions based on parameters fixed in 1985 and recent government data fully justifies our 2% accuracy. If the CEGB is able to produce quantitative information to support its assertion<sup>17</sup> that our calculations are "incorrect" it should do so in the normal scientific manner.

The CEGB response also put great emphasis on the statements made by ministers to parliament. The "encapsulation" of these<sup>18</sup> is that made by Mr Moore on 4 February 1983 (ref. 19) in which he said that "no plutonium produced in any of the CEGB's nuclear power stations has ever been used for military purposes. . . ." Yet in April 1986 the prime minister was only prepared to confirm this assurance for the period since 1979<sup>1</sup>. Energy ministers have repeatedly refused to say whether or not the earlier Moore statement is valid. The government assurance on which the CEGB relied in their response to our paper no longer appears valid for the first 16 years of CEGB Magnox operation.

Clarification by government and industry of the past history of civil plutonium production and end use, which can be done without compromising national security<sup>16</sup>, is now urgently necessary for the following reasons:

(1) Our original conclusion that more than 2 tonnes of civil plutonium are unaccounted for, over and above that known to be in the United States, is now strengthened by the tests described here.

(2) Important assurances about civil plutonium made to parliament and to the Sizewell Inquiry have, in effect, been invalidated by statements from the prime minister.

(3) The rounding of the recent plutonium production figures to the nearest 50 kg (equivalent to 10 warheads per station per year) in no way meets the Sizewell Inquiry inspector's request for "full and accurate" records<sup>11</sup>. Such accurate records are unlikely to be provided until the past history is clarified.

(4) The Magnox reactors are approaching the end of their working life. In their shut-down phase they will produce large quantities of military quality plutonium,

as they did in their early years. It is essential that before then plutonium production data is published in sufficient detail to allay suspicions of diversion.

(5) Publication of plutonium production figures in greater detail would be a better demonstration to the rest of the world that the United Kingdom is complying with the ideals of the non-proliferation treaty.

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## HLA antigens and insulin-dependent diabetes

SIR—Klitz states (*Nature* 333, 402; 1988) that the HLA class II DQ  $\beta$ -chain residue 57 correlation<sup>1</sup> does not take into account the complexity<sup>2</sup> of the association of HLA class II antigens with insulin-dependent diabetes mellitus (IDDM). On the contrary, this correlation explains most of these complexities: of 17 common Caucasian DR-DQ haplotypes, 16 are consistent with the view that predisposing haplotypes encode a non-charged amino acid at position 57 of the DQ  $\beta$ -chain and non-predisposing (neutral or negative association) ones encode Asp 57.

The exception is DR7, which is associated with a DQ  $\beta$ -chain that has Ala at position 57 and therefore should be positively associated with IDDM, but instead is neutral. This may be explained by the presence of the DR7 DQ  $\alpha$ -chain, which is found only on DR7 haplotypes<sup>1</sup>. In a recent model of class II structure<sup>3</sup>, it is suggested that  $\beta$ -chain Asp 57 forms a salt bridge with  $\alpha$ -chain Arg 79, occluding one end of the putative antigen-binding site. If this interaction is important in DQ Asp 57-associated IDDM resistance, variation in the  $\alpha$ -chain, as well as the  $\beta$ -chain, would affect disease susceptibility<sup>1,3-5</sup>. In support of this, DR3,4 individuals are at the greatest risk for IDDM<sup>6</sup>, implying that disease susceptibility is encoded by two different and complementing genes on the DR3 and DR4 haplotypes<sup>2</sup>. We have proposed<sup>5</sup> that, in addition to the DQ Asp 57-negative status of DR3 haplotypes, the DR3 DQ  $\alpha$ -chain also contributes to IDDM susceptibility,

by forming a hybrid molecule with a DR4 DQ  $\beta$ -chain. DQ $\alpha$  and DQ $\beta$  genes are therefore primary IDDM susceptibility genes which encode a number of distinct DQ molecules that regulate autoimmunity to differing degrees, producing the observed "scale of susceptibility".

More than 90 per cent of Caucasian sporadic<sup>1</sup> and familial (P. Morel *et al.*, manuscript in preparation) IDDM patients possess two DQ Asp 57-negative alleles. This includes DR4/X (X is not DR3 or DR4) patients. We suggest that DR4 is not 'dominant' but is increased in frequency because it is associated with the most predisposing DQ Asp 57-negative molecule (DQw3.2).

The three additional points raised by Klitz are also inaccurate: (1) the DR7 DQ  $\alpha$ -chain is not shared by DR4 haplotypes<sup>1</sup>; (2) we emphasized that 10 per cent of the patients studied had one DQ Asp 57 allele, implying that DQ Asp 57-mediated resistance was not complete and susceptibility could not be 'strictly recessive'<sup>1,5</sup>; (3) we did not exclude a role for DR in IDDM. The experiment by Nishimoto *et al.*<sup>7</sup> is equivocal because an IDDM resistance gene from the B6 mouse strain may have been present on the same chromosome into the *I-E $\alpha$*  transgene had inserted. Wicker *et al.* have demonstrated a role for the *I-A* region in IDDM regardless of *I-E* expression<sup>8</sup>. Non-obese diabetic mice directly transgenic for *I-A* and *I-E* genes will be required to determine more precisely the role of class II in IDDM.

Recent studies of HLA antigens and IDDM in different ethnic groups support a DQ correlation<sup>9-11</sup> (J. A. T., unpublished data). Other genes and environmental factors are likely to be involved in IDDM because only 2 per cent of DQ Asp 57-negative individuals develop the disease. But the DQ correlation is compelling and indicates a major role for DQ in IDDM. It is also subject to experimental verification.

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## Sexual selection in guppies called into question

SIR—Breden and Stoner<sup>1</sup> attempted to test an important prediction from recent sexual-selection models<sup>2,3</sup>. Their results may be consistent with the hypothesis that selection by predators on colour patterns of male guppies has led to a correlated evolutionary response in female mating preferences, but, as Endler<sup>4</sup> points out, alternative explanations are not excluded. Here, I discuss some of the points made by Breden<sup>5</sup> in his reply to Endler's criticisms, and raise some additional issues.

The existence of genetic differences in female mating preferences, suggested by Breden and Stoner's data, is further supported by my study<sup>6</sup> of two different guppy populations. Females from the brighter (more orange) population appear to prefer bright males but females from the more drab population have no apparent preference, rather than a preference for drab males as suggested by Breden and Stoner's data. Both of these studies are in contrast with the results of a field introduction of a high-predation guppy population to a low-predation locality by Endler<sup>7</sup>. The change in male colour patterns after the introduction suggests that females in the original high-predation population prefer bright males, although the strength of the preference could have been less than in low-predation populations. The differences between these studies suggest that female preferences can differ in degree as well as in direction.

Breden and Stoner argue that their data on female behaviour provide evidence for mating preferences. Endler suggests that the females could respond to the models as though they are predators; other possibilities are that females respond to the models as potential sources of food or recognize the dull model as a female rather than as a male. Breden states that females perform 'move-toward' behaviour<sup>8</sup> directed at the models, indicating that the responses are sexual, but it is not clear if my criteria<sup>6</sup> would accurately distinguish sexual responses from other forms of directed swimming when females and males or models are separated by partitions. Comparisons of the responses of female guppies to other females, males, models, predators and other stimuli in partitioned and open aquaria are needed to determine whether the technique gives clear-cut evidence of female choice.

Even if Breden and Stoner's data do reflect differences in female mating preferences, their results could arise through processes other than correlated change in female preference in response to direct selection on male colour patterns. Any change in mating preferences could lead to a corresponding change in colour patterns, leading to a similar prediction of correlated variation in female preferences and male colour patterns across popu-

lations. The possibility that selection on females could act through risk of predation while close to a bright male should not be rejected without testing; other direct effects are possible. Selection against hybrids between different locally adapted populations (outbreeding depression) could favour females that prefer males from their own population. This could include the adaptation of colour patterns to local predation regimes, as in Breden and Stoner's model, but local adaptation unrelated to predation could also favour females that prefer local males. Direct selection unrelated to sexual selection could lead to differences in the visual system of females that could affect mate choice. Differences in the visual environment<sup>4</sup> between streams could lead to biases in female choice. Female preferences might also diverge in separate populations as the result of random events during independent processes of sexual selection<sup>2,3</sup>.

Breden and Stoner's argument that differences in female preferences have resulted specifically from differences in predation regime depends upon a comparison of only two high-predation and two low-predation populations, not a sufficient sample to warrant such a strong generalization. Differences in female preferences resulting from any of the processes described above could produce the observed results by chance. A substantial amount of colour pattern variation among populations appears to be unrelated to differences in predation; our unpublished data suggest this may be especially true of the particular populations studied by Breden and Stoner. It will be necessary to compare preferences in many more populations with known predation level and known colour pattern distribution, and to validate the experimental techniques, before we will know if the conclusions of Breden and Stoner are correct.

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## Spot the mistake

SIR—There must be something wrong with Voorhees and Poggio's image analysis (*Nature* **333**, 364; 1988); their picture of a 'leopard' is, in fact, of a cheetah.

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## Cystic fibrosis and chloride-secreting diarrhoea

SIR—In commenting on Rotter and Diamond's article<sup>1</sup> on principles by which recessive alleles are maintained at high frequency, Pritchard proposed<sup>2</sup> that the cystic fibrosis (CF) allele is increased in frequency because of increased fertility of men carrying a single allele. The frequency of the CF gene (1/20 in Caucasians) is too high to be sustained by mutations alone (see ref. 3). Conclusions about the maintenance and increase on the CF gene must take into account data on the biochemical basis of this disease.

Since the finding<sup>4</sup> that CF sweat ducts have a decreased chloride permeability, much effort has led us closer to identifying the basic defect in CF. Two recent reports<sup>5,6</sup> show that the chloride channels of the apical membrane in airway epithelial cells of CF patients does not open, even though they are known to be present, when stimulated by the catalytic portion of the cyclic AMP-dependent protein kinase. This suggests that, in CF, the apical Cl<sup>-</sup> channels (or regulatory units) cannot be phosphorylated or do not respond. Although these results are obtained from airway cells, the same defect is probably present in other epithelial cells of affected organs such as pancreas or intestine.

Among the diseases that could produce a selective pressure are diarrhoeal diseases mediated by toxin-secreting bacteria. Two of these toxins, *Escherichia coli* LT (heat-labile) and cholera toxin, mediate their diarrhoeal effect through an increased level of cyclic AMP followed by a Cl<sup>-</sup> secretion, particularly in the crypt cells<sup>7</sup>. This secretion could be mediated by the apical Cl<sup>-</sup> channels defective in CF. Heterozygotes for the CF gene probably express 50 per cent defective cyclic AMP-dependent Cl<sup>-</sup> channels as are the defective proteins in other recessive diseases. CF heterozygotes should thus have fewer Cl<sup>-</sup> channels that could be opened and suffer less fluid losses on these types of diarrhoeas. This could have given CF heterozygotes a selective advantage when diarrhoeas caused many deaths in Europe and explain the high frequency of the CF gene found today.

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## Tree-ring carbon-isotope ratios re-examined

SIR—The tree-ring  $^{13}\text{C}/^{12}\text{C}$  ratio has been used as an indicator of historic climate and/or atmospheric composition despite the difficulties in interpreting the results due to other competing effects. Stuiver and Braziunas<sup>1</sup> have reported a  $\delta^{13}\text{C}$  gradient in 1850 tree-ring cellulose (from conifers). Their results show that  $\delta^{13}\text{C}$ , the carbon-isotope ratio relative to a standard, increases from  $-19\text{‰}$  near  $35^\circ\text{N}$  to  $-24\text{‰}$  at  $65^\circ\text{N}$ . (The  $\delta^{13}\text{C}$  value is more positive when the sample is enriched in  $^{13}\text{C}$ .)

Stuiver and Braziunas also reported similar dependencies with latitude (N or S) in cellulose and wood from deciduous and coniferous modern tree rings (although the deciduous trees are about  $3\text{‰}$  more negative than the conifers). With respect to the latitudinal dependence, the authors suggest "relative humidity and temperature appear to be the controlling factors, with the former the leading candidate".

Here we propose an alternative hypothesis for the latitudinal gradient. But first, we make two general comments on the original conclusions: (1) models predicting the isotope composition of dry matter in C3 plants show that the  $\delta^{13}\text{C}$  is a function of the ratio of intercellular to ambient  $\text{CO}_2$  concentrations,  $\text{Ci}/\text{Ca}$  (ref. 2). The effect of environmental stresses and physiological influences will be via differential effects on assimilation rate and leaf conductance that result in variations in this ratio. There are many empirical studies on the influences on  $\delta^{13}\text{C}$  in plant material (including the latitude, altitude and metabolism effects suggested by Stuiver and Braziunas) which should be re-examined from this perspective.

(2) Significant variations have been observed in unstressed plants both among species and among cultivars within species possessing the C3 photosynthetic pathway. The reported latitudinal variation in  $\delta^{13}\text{C}$  results primarily from the mean differences between species, with each species exhibiting a scatter of about  $1\text{‰}$  around the mean. The one exception to this is provided by a single high-latitude Douglas fir, which is around  $1\text{--}2\text{‰}$  depleted in  $^{13}\text{C}$  compared with the mean of mid-latitude Douglas firs. Similarly, for other tree-ring  $\delta^{13}\text{C}$  data summarized by Stuiver and Braziunas, the range for any one species seems to be about  $2\text{‰}$  (with no information on selection criteria). Thus the available information, although exhibiting a  $\delta^{13}\text{C}$  dependence on latitude, does not permit a clear distinction between possible genetic and environmental causes.

Nevertheless, because of the potential importance of tree-ring isotope measurements to studies of the palaeo-environment and, for example, to contemporary

forest ecology, we propose one environmental mechanism which provides a latitudinal variation in  $\delta^{13}\text{C}$  of the sign and magnitude observed.

In terms of the bulk of carbon assimilated by the coniferous trees studied by one of us and colleagues<sup>3</sup>, the dominant influence on  $\delta^{13}\text{C}$  was light level. To sustain an argument that this light-dominated mechanism applies to the latitudinal-gradient data of Stuiver and Braziunas, it is necessary to demonstrate that light is a growth-limiting factor for the species involved, over the relevant latitude range ( $35^\circ$  to  $65^\circ\text{N}$ ).

Using data from the world actinometric network, Budyko<sup>4</sup> computed values for the photosynthetically active radiation for the 'vegetation season' for different regions of the Soviet Union. Using these data, and including the influence of temperature, turbulent exchange and respiration, he estimated potential vegetation yields (amount of assimilated  $\text{CO}_2$  per unit area) as a function of latitude (moisture non-limiting). The strong dependence in potential yield, going from  $70^\circ\text{N}$  to  $40^\circ\text{N}$ , is attributable largely to radiation flux. In the sense that the variation in  $\delta^{13}\text{C}$  in the leaf model represents a kinetic fractionation (depending on the rate rather than the amount of assimilation), the results of Budyko are more appropriate here than actual measurements of yield, in which many time-related and tissue-capacity factors can limit growth.

Budyko's 'light curves' exhibit appreciable slope (assimilation dependence) below about  $0.3\text{ cal cm}^{-2}\text{ min}^{-1}$ , which is equivalent to a photosynthetic photon-flux density of around  $400\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$ . Photosynthetically active radiation over the vegetation season ranges from  $70\text{ kcal cm}^{-2}$  at  $35^\circ\text{N}$  to  $20\text{ kcal cm}^{-2}$  at  $65^\circ\text{N}$ . These represent similar changes to those which produced observed  $\delta^{13}\text{C}$  changes of around  $4\text{‰}$  in individual trees<sup>1</sup> (in agreement with model prediction if translocation effects are assumed). These, in turn, are of similar sign and magnitude to the reported tree ring  $\delta^{13}\text{C}$  difference of  $4\text{--}5\text{‰}$  over this latitude range<sup>1</sup>.

From these observations, it seems that radiation level could be major factor in determining the average tree ring  $\delta^{13}\text{C}$  in the mid-to-high latitude bands, possible outweighing genetic differences for some species. The converse argument is that the average  $\delta^{13}\text{C}$  of tree rings indicates potential yield.

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STUIVER AND BRAZIUNAS REPLY—As we noted<sup>1</sup>, the  $^{13}\text{C}/^{12}\text{C}$  offset between tree cellulose and atmospheric  $\text{CO}_2$  can depend on many environmental parameters, such as precipitation, temperature, physiology, light intensity, nutrients and relative humidity. As a possible interpretation for the demonstrated latitude dependency of  $\delta^{13}\text{C}$  of trees, we focused on relative humidity and temperature. Francey and Hubik, although concerned about possible genetic influences on the latitude relationship, suggest that the controlling factor is light intensity. Latitude-dependent light intensity, as well as nutrient availability, indeed should contribute to the observed relationship. But we are not convinced that light intensity overrides all other components of this complex system.

Support for the dominance of light intensity of  $\delta^{13}\text{C}$  is taken from the work of Francey *et al.*<sup>3</sup> on physiological influences on carbon-isotope discrimination in Huon pine. A  $1\text{‰}$   $\delta^{13}\text{C}$  difference of  $6.6\text{‰}$  is calculated between a sunny and shaded branch of Huon pine, whereas the observed difference is  $2.1\text{‰}$ . The authors assume translocation to be the attenuating factor, but do not offer independent experimental evidence (such as the use of  $^{14}\text{C}$  tracer) that provides the magnitude of inter-branch translocation for Huon pines. For red pine, each branch appears to be in a large measure self-supporting<sup>5</sup>.

Extrapolating the Huon pine study to other species is problematical. Huon pine has a stomatal insensitivity to light, whereas most species exhibit substantial response to light<sup>2</sup>. There is no evidence of a humidity response of Huon pine needles, a factor possibly related to the suggestion of unusually low stomatal conductances observed in field Huon pine compared with those of most species<sup>2</sup>. Evidently, the anomalous genetic make up of Huon pine could result in a  $\delta^{13}\text{C}$  response substantially different from that of most other species.

Light intensity is an important factor influencing  $\delta^{13}\text{C}$  in trees<sup>3</sup>. However, the proposed quantitative framework for interpretation of a latitude relationship dominated solely by light intensity may be too limited.

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# Controversial recollections

John Morton

**The Invention of Memory: A New View of the Brain.** By Israel Rosenfield.  
Basic Books: 1988. Pp.229. \$18.95.

IN THE *New York Review of Books* of 9 October 1986, there appeared an article extolling the virtues of the work of Gerald Edelman. The subject matter of the article ranged from the immune system, through cell adhesion molecules and neural groups up to the Bartlettian idea of memory as reconstruction. This latter theory is given "a precision and a physiological justification" by Edelman's work, we were told. I wasn't convinced by this claim, but I was impressed by the final sentence: "Humanism never had a better defense".

The article was by Israel Rosenfield and it is reprinted, virtually unchanged, as the final chapter of *The Invention of Memory*. The rest of the book is made up of a number of digestible segments of psychology and neuropsychology, showing how everyone else is wrong in their approach to memory, and leaving the way open for Edelman to come riding to the rescue in the final reel. On the question of what it is that Edelman is riding on, Horace Barlow (*Nature* 331, 571; 1988), in reviewing Edelman's own book, generously invited his readers to decide for themselves "whether it ushers in a new era in neuroscience or whether it's just a hopeless muddle". (A response to that review, together with a counter by Barlow, appeared in *Nature* 332, 787; 1988.)

To people unfamiliar with developments in cognitive psychology, Rosenfield's potpourri may have the smell of righteousness about it. But the target of his indignation, the "invention" of the title, is a construction. What Rosenfield has done is to string together a number of themes as if they were the mainstream

theory. The prime opponent is that memories are permanent. The best recent attack on this belief, by Loftus and Loftus (*Am. Psychol.* 35, 409-420; 1980), concluded that there was no good evidence in favour of it. The authors also pointed out that there is no good evidence against it either. Rosenfield's approach is to link the permanence of memories with other propositions which seem more vulnerable. Attack some of them, and they all fall. Guilt by juxtaposition, you might call it. Non sequiturs are heaped up into an enthralling pile:

What we see depends not on any computations but on what we have seen and experienced in the past as well as in the immediate present . . . our experience, feelings, and thought differ widely from person to person and these differences cannot be accounted for by any processes as rigid as computations [p.8].

The reason that Rosenfield is against 'computation' is as mysterious as his arguments. Still, I hope it is clear that humanism is in good hands.

The book is divided into four parts. The first part is devoted to an engaging enough attack on the concept of localization of function. We get case histories from the second half of the last century which are punctuated by assertions such as ". . . the brain does not sort information; if it did, context would be irrelevant to perception" (p.66). "Recollections without affect are not recollections" (p.72). "In a sense, all acts of recognition, all acts of recollection, require some kind of motor activity" (p.78). Thank goodness for that "in a sense". And how about ". . . it

would be misleading to say that a pianist's rendition of a sonata is a recollection of an earlier performance" (p.80). Yes, yes, except "in a sense".

What does all this have to do with localization? The idea is that all recollection is both context dependent and reconstructive. Any evidence in favour of these, unexceptional ideas is taken as evidence against localization, against modularity (that is, the basis of contemporary cognitive psychology — that we can meaningfully divide up brain processes into component functional parts) and against computational approaches to the mind. Unfortunately, Rosenfield has failed to note that modularity of function does not imply localization of function. More unfortunately, he has not fully mastered all his subject matter. For example, Marshall and Newcombe (*J. psycholing. Res.* 2, 175-199; 1973) establish that their deep dyslexic patient could not read prepositions out loud but could correctly pick out a picture described by a sentence such as "The cup is *under* the table". Their point is that this patient can only achieve a partial understanding of words like *under*; insufficient for reading aloud but sufficient for making a simple decision. Rosenfield ploughs in with the grandiose "Like much of contemporary psychological work, these studies have ignored the effects of context" (p.93), proceeding to treat both tasks equivalently as *recognition* and thereby abolishing 20 years of careful, context-appreciating distinctions at a stroke.

The middle sections deal with speech perception and visual perception. The general theme is that perception is influenced by context, therefore it is "fluid", therefore memories cannot be fixed. The pervasive confusion in these sections between perceptual structures and (cognitive) memory leads to some amusing errors. Thus, Rosenfield understands Marr's theory of vision as implying that perception is independent of past experi-



Death mask — a specimen of the toad *Bufo marinus*. The picture is taken from *Illuminations: A Bestiary*, a book of colour photographs by Rosamond Wolff Purcell, with accompanying text by Stephen Jay Gould, in which photographs of preserved specimens cast new light on their anatomy and evolution — "Death strips information from an organism, layer by layer". The book was first published by Norton in 1986, and is now available in paperback, price \$26, £14.95.

ence. He blunders to the attack: "What we see in a painting, for example, is deeply affected by our cultural background" (p.135). But the level which is experience-free for Marr in no way corresponds to the kinds of processes involved in interpreting a painting. It is as if Rosenfield has mistaken the nature of Marr's 'primal sketch' and thought it relevant to art! In a similar vein, Rosenfield claims that anyone who learns a foreign language will "be able to categorize speech sounds in new ways" (p.111). But, for example, adult Japanese cannot learn the *ra-la* distinction which is missing from their native tongue. Some phonetic distinctions are fixed.

What does all this have to do with Edelman or Edelman with the psychology of memory? Not a great deal, I fear. If Edelman's neural groups have enough power to instantiate any one psychological theory of memory they will have the power to instantiate the others. Debates concerning the structure of cognitive processes cannot be decided at the level of the

cell. This, final, reductionist error of Rosenfield's is a fitting climax to a wonderfully confused book.

Perhaps I have been too hard. The writing does have a certain gloss to it; Rosenfield writes of Sigmund Freud as if they had been chatting in a café only last week. And on the back of the dust jacket you may read Oliver Sacks: "A remarkable synthesis of the most important developments in neurobiology today". Jonathan Miller says "Israel Rosenfield has an admirable, imaginative grasp of leading issues in the neurosciences today". Finally, one John Marshall writes a short essay, ending with "The approach holds considerable promise of throwing light upon the psychobiology of human diversity and our exquisite adaptations to the ever-changing flux of individual experience. Read, reflect, enjoy!". At least he didn't say "Believe!". □

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## Solid-state success

Andrew Holmes-Siedle

**The Conquest of the Microchip.** By Hans Queisser. *Harvard University Press: 1988.* Pp. 185. \$24.95. To be published in Britain in July, £19.95.

HANS QUEISSER, then a young academic, left Germany for the United States in 1959. He observed the development of the microchip from two vantage points. First, that of a job with William Shockley, the inventor of the transistor, in California; and later as an employee of Bell Laboratories, where he distinguished himself with his research on electronic devices made of silicon. Spinoffs from Shockley's firm produced the research and business complex which has come to be known as Silicon Valley. Now back in Germany, Queisser has distilled his personal view into a short book. A narrative account of the history of semiconductors, it is interspersed with observations on the state of the semiconductor industry around the world and brief examples from the author's own experience.

The book was first published in German and I assume that Queisser himself has rewritten the text in English, while bringing the account right up to date. The result is a lively story which only occasionally suffers from compression of complex physical concepts into a couple of sentences. It is not an autobiography, and the author reveals little of his own life. He is, however, not afraid to draw lessons from what he has seen and to reflect on what might have been.

Queisser sees the period from 1910 to 1940, in which the vacuum triode was supreme in electronics, as a massive diversion from the inexorable march of solid-state electronics. There was a crucial time after 1910 when a solid-state triode might have been produced in Germany. The mind reels at the possible alternative courses of history.

As a European who has seen the development of electronics in many countries, Queisser can make valuable observations on why the United States and later Japan reaped many of the fruits of solid-state research which had originated in Europe. For example, Walter Schottky of Siemens in Germany gave a brilliant explanation of the current flow at interfaces between a semiconductor and a metal — a puzzle that had effectively held up solid-state physics for 30 years. Nevertheless it was at Bell Labs that the knowledge was exploited and then efficiently communicated to American firms. Queisser is merciless in criticizing the cultural traits of his own country which led to poor communication and lack of technical vision. For example, in German academic circles it seems that at one time it was almost unheard of for a theoretician to talk to an experimentalist.

Politicians, too, get their broadside: in describing the current attitudes of Japanese administrators, Queisser says "How different from American and European politicians who carefully and proudly try not to acquire the appearance of a scientific specialist or even an amateur!". This criticism is certainly valid in Britain, where politicians are rarely recruited from the scientific community. A recent speech by Ian Barron of Inmos, extracted in *Electronic Times* of 24 March 1988, criti-

cizes the "failure of nerve" of British industrialists when investment is required. It is more than likely that the failure of nerve comes from lack of knowledge.

The sentence from the book just quoted illustrates a characteristic of Queisser's writing — the phrasing is blunt, and even inelegant, but never bereft of meaning. The author is looking for ironies and frequently finding them: the fact that an element called germanium was instrumental in the defeat of its namesake in the air war over Britain in the 1940s; and that the sublime study of the crystal has ended up making a tool for building computer games. In viewing recent history, the author senses tragedy for the West. He demonstrates that American industry, plagued by poor policy decisions, is withering inexorably under a brilliantly directed 'semiconductor war' pursued by the Japanese.

I have only mild objections to Queisser's image of the 'crystal' as the key to all success in semiconductor integrated-circuit development. We now buy, off the shelf, exactly the quality of silicon crystal we want. The leaders in the field of integrated circuits are there largely because they have developed better processing techniques. Of course good silicon is vital; but patterning of photoresist, etching, and oxide and nitride formation depend more on good apparatus and mask design than on silicon perfection. On the other hand, the author is right that no other substance, even water, has been more thoroughly investigated than the silicon crystal; silicon, as an engineering material, indeed has few rivals.

I read this book from the viewpoint of someone who once worked for a solid-state firm not many miles from Bell Labs; because of that I was able to follow the author's shorthand easily. Others may struggle a little but the significance of the subject (material for several books and hundreds of PhDs) and the penetration of Queisser's account make any such struggle richly worthwhile. □

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### New in Britain

• *Bones of Contention: Controversies in the Search for Human Origins* by Roger Lewin, which demonstrates how scientists' preconceptions are at least as important to their interpretations as the fossils they are examining. Publisher is Simon & Schuster, price is £14.95. For review see *Nature* 330, 277 (1987).

### New in English

• *Murderous Science: Elimination by Scientific Selection of Jews, Gypsies, and Others, Germany 1933-1945* by Benno Müller-Hill, translated from the German by George R. Fraser. Publisher is Oxford University Press, price is £15, \$24.95. For review see *Nature* 313, 407 (1985).



# The mobilization of science

Willem Hackmann

**The War of Invention: Scientific Developments 1914-18.** By Guy Hartcup. *Brassey's*: 1988. Pp. 226. £24, \$43.

IN A characteristic memorandum written shortly before the First World War, an exasperated Sir John Arbuthnot (later Lord) Fisher vented his spleen over the Admiralty's inability to grasp the potential importance of the submarine in warfare: "There is a strong animus against the submarine — of course there is! An ancient Admiralty Board Minute described the introduction of the steam engine as fatal to England's navy. Another Admiralty Board Minute vetoed iron ships, as iron sinks and wood floats".

The submarine and other new technologies were widely regarded with suspicion by the pre-War military establishment — another admiral, A.K. Wilson, described the submarine as "underhand, unfair, and damned un-English". The meddling of scientists in matters of warfare was resented by the military establishment and senior civil servants, and there was also the question of status. What was the social rank of a scientist? Even the term was relatively new, and for most people there was little to choose between a 'scientist', an engine-driver and a man who repaired machines. The question of the standing of the civilian scientists and engineers in the Royal Navy remained a hotly debated issue for many years, until the Royal Navy Scientific Service was firmly established.

As Hartcup's book shows, the First World War had a dramatic impact on military scientific research and development. The general assumption was that the war would be over quickly. But when it became apparent that this would not be so, it became imperative to explore all avenues — even science — in order to stop the German war machine. In the words of H.G. Wells, the war had become a "struggle of invention", and many of the country's senior scientists began to complain that their expertise was not being used to good effect.

The need to manufacture the chemicals, optical glass and other products that could no longer be imported from Germany resulted in the setting-up of the Advisory Council for Scientific and Industrial Research in July 1915, which, as the Department of Scientific and Industrial Research, remained until the 1960s. Shortages could lead to desperate solutions. Nearly 1,000 tons of alcohol, or the equivalent of 200,000 tons of proof spirit, were required for the production of a new variety of cordite, which led to the restric-

ted sale and increased cost of whisky. A bizarre tale told by Hartcup concerns the pressing requirement for field glasses in 1915, which compelled the War Office to open secret negotiations with the German government for the supply of some 32,000 binoculars through a Swiss intermediary, in exchange for rubber.

This slim volume is a synoptic and readable account of how an entire scientific community was mobilized for war work for the first time in history, from the War Committee formed by senior scientists at the Royal Society in November 1914 to the plethora of committees and sub-com-

parison between the military research organization, and the scientific response to war developments by the Germans and by the Allies, although this subject still requires a deeper analysis for both world wars. Hartcup deals succinctly with the organizational aspects and with the 'fruits' of the resulting research. British wartime landmarks were pioneering work in explosives (the rapid development of TNT, Amatol and improved armour-piercing explosives), aerodynamics, underwater acoustics (for detecting submarines), chemical warfare and the treatment of infectious diseases. Many of the actual

IMAGE  
UNAVAILABLE  
FOR COPYRIGHT  
REASONS

*A fish hydrophone for detecting U-boats is lowered into the sea, October 1917.*

mittees which, by 1918, were dealing with all aspects of scientific warfare. The first idea was to establish a mechanism to evaluate the thousands of unsolicited inventions that poured into the various government departments. The Admiralty's Board of Invention and Research (BIR) — also known in the navy as the "Board of Intrigue and Revenge" because it was headed by the outspoken Lord Fisher — was swamped with some 100,000 inventions, of which perhaps 30 could be developed. The Munitions Inventions Department received 47,949 and the Air Inventions Committee some 5,000, but the number that turned out to be useful was again small. Similar statistics emerge from France and the United States. It was soon realized that the way forward was for scientists to become involved in research towards specific goals. As the author points out, the scientists who were most valuable to the war effort were those who were able to appreciate the nature of a problem quickly and to suggest a method of solving it.

An interesting aspect of the book is the

instruments of warfare had their origins in the decade or so before the war, but their development was accelerated dramatically as a result of war research.

This book illustrates — although perhaps does not emphasize enough — that the development of wartime technologies is governed by an intricate web of factors: scientific, bureaucratic, tactical and political. It covers the scientific developments of the main protagonists in the First World War, and is best on British events. Military research is a mixture of fundamental science and research and development; the goal is not the elucidation of a basic principle, but the development of an operational device. During the First World War, a large number of civilian scientists, engineers and technicians were introduced to war research for the first time. Those who remained in service after the war formed the core of what has evolved into Britain's present-day military research complex. □

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# Acorns and geese after Darwin

A.J. Underwood

**Barnacle Biology.** Edited by Alan J. Southward. A.A. Balkema: 1987. Pp.413. £47.

CHARLES Darwin's monographs on barnacles remain standard works on this widespread and highly modified group of crustaceans. Now, in *Barnacle Biology*, new studies have been brought together with the various aims of replacing, complementing or simply adding footnotes to Darwin's interpretations.

Here are to be found current opinions on the phylogeny and origins of the higher taxa (families and suborders). The available palaeontological evidence is summarized in a readable chapter and there are contributions on larval form and function; that on balanomorph (acorn barnacle) larvae is likely to prove particularly useful for teaching. We are also given a detailed and readily interpretable review of feeding and other functions of the cirri (modified appendages). It is notable that comparative zoologists still have a considerable part to play in integration of the evolutionary, palaeontological and ecological strands into a vision of form and function of extant animals.

The sexual antics of barnacles prompted Charnov to develop models to account for their various types of reproduction. He discusses them in his chapter. Austin and Dando review methods of analysis of barnacles' small and difficult chromosomes, and the use of polymorphic enzymes to shed light on hermaphroditism, self-fertilization and taxonomic problems.

More practical aspects — how barnacles settle and adhere, and how to prevent both activities — are covered in sections on fouling. Gregarious settlement by larvae has often been reviewed previously; here, the chemistry of the proteins responsible for inducing settlement is given prominence, as is the role of proteinaceous adhesives left by cyprids when they are exploring a surface before they finally settle. Both are important in determining how, where and when barnacles will settle, and discussion of them leads to a good account of the application, mechanisms and effectiveness of anti-fouling treatments.

There are reviews of many other aspects of the biology of barnacles. These contributions fall into two groups. The first, covering such topics as the circulatory system, lipid metabolism, structure of shells, endocrinology and excretion, provides comparisons between barnacles and other crustaceans. These are a mine of useful

information; anyone interested in the ecology or general biology of barnacles will want to read them. The second group, of two chapters only, is concerned with the neuromuscular system. These articles tell us not so much about barnacles themselves, but a great deal about how useful barnacles are for learning about other types of animals.

The two chapters on ecological relationships, and settlement and competition, are disappointing. They miss the variety of non-linear interactions of barnacles with other animals. The alternative outcomes of such interactions are emerging from recent experimental studies of the complex roles that barnacles have in the structure of intertidal assemblages. More critical evaluation of the worth of experimental ecological data would have made these chapters much better; for example neither

of them discusses the consequences for ecology (of barnacles and, possibly, generally) of recent models formulated by Roughgarden and his co-workers.

The book is dedicated to J. H. Crisp. Such *Festschriften* are often patchy, but in this case Southward has done a splendid job of editing. The volume is comprehensive and well organized, and each author seems to have been given a well-defined brief. Non-ecologists can learn a great deal from it; ecologists can learn even more, because the authors provide so many good syntheses outside that field. Darwin's monographs have apparently been replaced by a worthy successor, albeit one unlikely to stand for so long the tests of time. □

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## Attitudes to AIDS

Richard S. Tedder

**The Search for the Virus: The Scientific Discovery of AIDS and the Quest for a Cure.** By Steve Connor and Sharon Kingman. Penguin: 1988. Pp. 230. £3.95.

WITH ITS implication that here is an account of the early work leading up to the realization that AIDS is a sequel to a specific infection, and of the subsequent identification of the infecting organism, the title of this book whets the appetite. Sadly, that appetite is likely to remain unsated.

It is not entirely clear at whom the book is aimed. Certainly, readers of popularized science, as exemplified by *New Scientist* for whom Connor and Kingman both write, will be at home with the depth and style of presentation. Parts of the book have already appeared in print, and the certain lack of fluency may be a result of an imperfect melding of the original manuscripts. In the first two chapters it is disconcerting to be continually moving backwards and forwards between 1981 and 1988, and dates in between, which makes it difficult to get a feel for the sequence of events and changing attitudes. A more specific criticism is that, as in their original articles, the authors take a partisan stance for the Pasteur Institute (Montagnier) and against the National Institutes of Health (Gallo) in the dispute over who discovered HIV. That dispute was more between patent agents than between the scientists, and both groups have over the past five years collaborated fully with their counterparts in other countries. That should not be forgotten.

The first 30 or so pages contain a useful if salutary review of the identification of the infectious nature of AIDS, whilst the following section, of the same length, is

devoted to an exposé of the Gallo-Montagnier affair. Finally purged, the reader emerges into the remaining two-thirds of the book.

Here the treatment is better balanced but somewhat superficial. The authors pay lip service to the idea that the serological test for antibody to HIV (anti-HIV) was unsuitable for diagnostic use because "the test was made in order to stop infected people from donating blood. . . . The use of the test as a diagnostic tool . . . was in fact a spin-off of this" (p.65). If anything, the blood-bank screening was the spin-off. More importantly, although Connor and Kingman imply otherwise, it is unlikely that the origins of the anti-HIV test had anything to do with the emergence of ethical problems related to its use.

The chapter on vaccines suffers not only from being removed from the chapter on virus structure, but again from shallowness. Thus it is stated that, alone, it is the variability of the virus that hampers production of a vaccine. This is one, but only one, aspect of the difficulty of producing HIV vaccines. A more robust and perhaps conventional account of vaccine and protective immunity (and the absence of it in HIV-infected persons) would have been of greater benefit for the non-specialist reader.

Parts of this book are interesting, but the interest at times borders on the prurient. For example, the descriptions of the techniques of obfuscation used by the authorities in the United States are not flattering and will come as a surprise to some readers. These days there is a plethora of AIDS books milling around on the starting line, and this one — though out of the stalls — is unlikely to be a winner. □

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# Canada's changing times

*Canada is looking to science as part of a formula that will bring future prosperity. But researchers question whether the government understands the nature of the scientific enterprise.*

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"Research is an act of enlightenment for society. When fiscal climates are tight, selling enlightenment is tough."

*Geraldine Kenney-Wallace, Chair, Science Council of Canada*

"People ask 'Why spend money to do fundamental research in Edmonton when they're already doing it in the United Kingdom, Germany and Japan?' We're fighting this all the time. We have to convince them we're as good as Germany or the United States."

*William Bridger, Chairman, Biochemistry Department, University of Alberta*



"A misunderstanding of the root sources of scientific progress can lead one to look for the quick technological fix for problems the solution of which can only come from a deeper understanding of basic science. The billions of dollars spent on the complex infrastructure for open heart surgery does nothing to eliminate heart disease. By contrast, when the National Foundation for Infantile Paralysis invested comparatively modest sums in basic research on viruses rather than on artificial lungs, the development of a polio vaccine became possible and the prevention and virtual elimination of polio at very low cost became a practical reality."

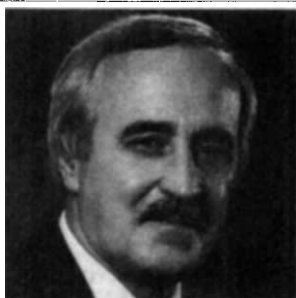
*Arnold Naimark, President, University of Manitoba*

"To support [university-industry] initiatives while largely ignoring the need for a strong network of free and fundamental enquiry is absolute folly as other nations have already discovered."

*Gordon MacNabb, President, PRECARN Associates and former President of NSERC*

"The average Canadian believes that if something is technically sophisticated, it clearly came from somewhere else. If it is made in Canada, it is clearly suspect. But in fact, Canadian technology is generally superior."

*John MacDonald, President, MacDonald Dettwiler and Associates Ltd*



"As far as Canada's science policy, it's hesitant in its character. There has been a lack of leadership at the top for 30 years. But now the government is giving more attention to science policy than it has for 25 or 30 years. The preoccupation may be short-term, but the advisory mechanism for the Prime Minister has been set up, and that may be the right thing."

*Gilles Cloutier, Rector, Université de Montréal*



[On science policy] "There is a cacophony of voices. It's a thriving time, an exciting time."

*Gordon MacLachlan, Dean of Graduate Studies and Research, McGill University*



"We don't honour science in this country and we don't have a culture of science."

*Charles Scriver, professor of biochemical genetics, McGill University*

"Does Canada have a science policy?' I am sure some of my fellow cynics out there are wondering whether I am going to have sufficient intestinal fortitude to give a one-word, two-letter speech, and then sit down. And I have to admit that I am tempted." (Speaking at the National Conference on University Research and the Future of Canada.)

*Gordon MacNabb*



"Even within the scientific community there are two populations: those who want to give the dynamic, productive groups what they need to stay in there, even if others have to lose funds, and those who think that everyone should get something."

*Dan Skup, Director, Montreal Cancer Institute*



"The politicians are looking for a quick fix to the lack of technology in industry. Had they funded biomedical research and allowed more world-recognized centres of excellence to develop we'd have more discoveries to exploit . . . the government is trying to enforce collaboration and collaboration should spring naturally. Trying to enforce an immediate industrial spin-off is not going to work and we are going to have a lot of unhappy politicians."

*Richard Cruess, Dean of Medicine, McGill University*

"Every political issue in Canada is ultimately a question of federal versus provincial power."

*George Connell, President, University of Toronto*

# Canada finds a name, but still searches for an identity

WITH a little bad luck, Canada could have ended up as Mesopelagia, Albionara, Cabotia, Efisga, Hochelaga or Tuponia.

These were among the names put forward for the new nation before confederation was achieved in 1867. Some, like Cabotia, were based on the names of early explorers, others were clumsy acronyms; Tuponia stood for The United Provinces of North America. But Canada it was, pleasing the original French settlers, who much earlier had taken the name from an Iroquois word recorded in the journal of the sixteenth century French explorer Jacques Cartier.

Confederation at first involved only the colonies of Upper and Lower Canada (now Ontario and Quebec), and the Atlantic Provinces of Nova Scotia and New Brunswick. Manitoba joined in 1869 and British Columbia two years later, but only after Ottawa had promised to build an east-west railway line. With the inclusion of the vast Northwest Territories, governed directly from Ottawa, the world's second largest nation had been created by 1871.

Canada might have been larger still. In the early eighteenth century, French territories extended in a ribbon from Labrador, along the St Lawrence River and down to the Gulf of Mexico, hemming in the developing American colonies.

The American War of Independence rewrote the map, but quarrels with the United States were to continue. Canada came close to annexation in the 1812 war between Britain and the United States. Oregon, which had been granted by the Crown to the Hudson's Bay Company, had to be ceded to the United States in 1846 when war threatened again.

Canada's proximity to the United States continues to dominate political life. Most of Canada's 24,500,000 people live in a narrow strip of land along the border. Although Canada has the seventh largest economy in the free world, its trade is tied to the United States. Canada and the United States are each the other's most important trading partner, but the relationship is not equal. Almost 80 per cent of Canada's exports go to the United States, as against 23 per cent of US exports to Canada. And most of Canada's exports are raw materials and energy while its imports are manufactured goods.

US companies have made massive investments in Canada. They own more than half of Canada's manufacturing, mining and smelting industries, almost all of its rubber and automobile industries, and most of its chemical and electrical industries. While investment has created jobs it has also made Canadians speak of

their country as just a "branch plant" (or worse, a "warehouse") for the United States.

For over a century, Canada has swung between accepting the dependence on the United States for the benefits it confers, and striking out for greater autonomy. Free trade treaties have come and gone, being replaced by tariff walls designed to build a Canadian identity. In 1911 a free trade treaty was thrown out in a general election because of fears that it would lead to a cultural annexation of the country by the United States.

The old fears of engulfment have not gone away. Now before the Canadian Parliament, and as bitterly contested as ever, is the most comprehensive free trade agreement ever considered. Its 1,400 pages have caused little stir in the US Congress but, as we go to press, are still being hotly debated in the Canadian Parliament. An election in the autumn could still lead to a repeat of the 1911 debacle.

If put into effect, almost all tariffs on goods and services will be abolished over a ten-year period. First advantages may go to Canada, which already has a US\$13 billion trade surplus with the United States and can look forward to marketing freely its agricultural produce and energy. But in the long term, Canada will only reap benefits from free trade if its industries can shape up to US competition.

The frustrations of living next door to a giant (like being a "mouse in bed with an elephant" as former Prime Minister Pierre Trudeau once described it) are brought home to Canadians by two other issues — the spread of pollutants that cause acid rain (see p.736) and US unwillingness to recognize Canada's full sovereignty over the Arctic (see p.733). In both cases

Canadians would like to get angry but have learnt that to make progress they have to talk slowly and patiently.

The division of powers between the federal and provincial governments is a second source of endless debate. At the time of federation, several forces were at work. The French-speaking, Catholic province of Lower Canada, later Quebec, wanted federation so as to gain greater autonomy; Upper Canada (later Ontario) wanted greater central control; both wanted to push west and all the colonies wanted a strong front to prevent annexation by the United States. The result, the British North America Act of 1867, created a federal government which regulated what were then seen as the key issues of national life: trade, commerce, defence, taxation, banking, inter-province transport, shipping and fisheries. Left to the provinces were education, health, resources and municipal institutions.

The greatest quarrel with federalism has come from Quebec, where a government committed to independence was elected in 1976 (see p.730). A new constitution emerged in 1982 when the old British North America Act was modified and made an act of the Canadian, rather than the British, parliament. But Quebec was not satisfied by the change and a series of negotiations, named after the Prime Minister's Meech Lake retreat where they began, attempted to accommodate Quebec's desire for recognition as a "distinct society within Canada" and for protection of French cultural minorities elsewhere in Canada. The form of the constitution has had one curious effect. Education was left to the individual provinces and, despite the enormous increase in its importance over the past century, that is the way it has remained. As a result educational standards are not uniform and there is great variation in the level of support individual provinces give to universities and scientific research. □



Canada's Prime Minister, Brian Mulroney (left) heads the Progressive Conservative party which holds a commanding majority in the House of Commons (208 seats) over the two major opposition parties, the Liberals (39) and the New Democrats (32). The Liberals, under Prime Minister Pierre Trudeau, were in power for much of the 1970s. A general election does not have to be held until September 1989 but there are rumours that an earlier date will be chosen, probably autumn this year.

Members of the upper house, the Senate, are appointed by the Prime Minister to represent particular provinces and territories, and hold office until they are 75 years old. The Senate largely concerns itself with tidying up legislation and has not rejected a bill for 40 years. The executive and legislature are not strictly separated as in the United States. The Prime Minister appoints cabinet members, virtually all of them from the House of Commons. The need to draw members from all the different regions means that the cabinet is extraordinarily large — 39 people at present — and that real power passes from full cabinet meetings to cabinet committees. □



# Underinvestment lies at the core

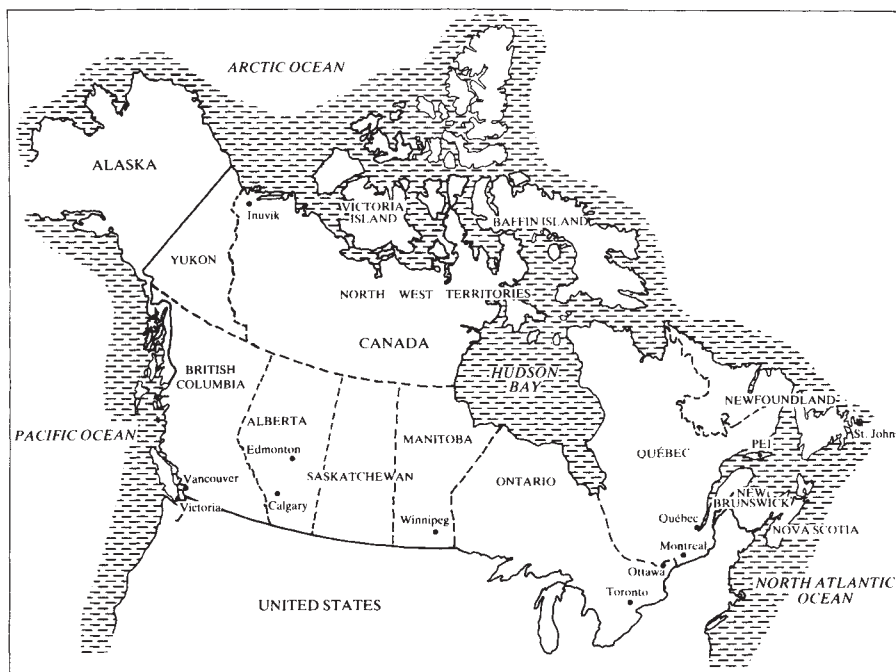
CANADA spends substantially less of its gross domestic product on research and development than any other large industrialized nation in the world. No matter how it is modified or recalculated to cast it in a better light, that fact still represents the departure point for understanding how Canadian science has arrived at its present state, and where it may go in the future.

Although it is fashionable to blame government spending for such shortfalls, responsibility in Canada's case is shared between government and the private sector. Government funded research and development is low by international standards, but industrial spending on research, at 0.57 per cent of GNP, is practically off the charts, only slightly more than half of the next lowest country, according to OECD statistics.

Some three quarters of Canadian firms have no research and development capacity at all, and a scant two to three per cent of the remaining quarter do the bulk of all industrial research and development in the country. Four companies — Bell Canada Enterprises, Atomic Energy of Canada, Ltd, Pratt and Whitney and IBM Canada — account for more than a third of the total, and of those only Bell Canada is neither a crown corporation nor foreign owned.

Two factors have been particularly important in arriving at this state of affairs. First, many of the largest Canadian companies that might reasonably be expected to conduct research are foreign owned. As 'branch-plants' they tend to eschew research in favour of marketing and distribution of goods, a decision that no doubt pleases corporate headquarters in New York, Paris or London, but one with devastating consequences for Canada's scientific enterprise.

Another reason for the history of low spending on research is Canada's great natural wealth. The easiest way to make profits has traditionally been to cut trees, grow wheat, or dig coal, and ship these raw materials to other countries where



they were assembled, baked or burned.

But even resource based companies spend less than their foreign counterparts on research. When, for example, Canada's forestry industry found that the supply of 'old growth' forests was not inexhaustible, there was no corporate expertise in silviculture that would easily allow them to plant new trees.

James Gilmour, research director for the Science Council of Canada, says there is a growing sense that Canada's future lies in harnessing its brains, but there is no consensus that the resource era is over.

If government is not primarily to blame for this industrial short sightedness, it has hardly been a force for change. By means of grants, research and development contracts, and tax breaks Canadian government supports approximately 20 per cent of the industrial research that is done in the country. But that is half what the US government spends in supporting industry, and well below the support industry receives from the government

in the United Kingdom, France and Germany.

## Government science

Where Canada does achieve parity with world norms is in the percentage of GDP devoted to research in government laboratories.

An estimated \$2,250 million is spent by the National Research Council and the

### Research and development as percent of GNP

|              |     |
|--------------|-----|
| Japan        | 2.8 |
| US           | 2.7 |
| West Germany | 2.7 |
| Sweden       | 2.5 |
| France       | 2.4 |
| Switzerland  | 2.3 |
| UK           | 2.2 |
| Netherlands  | 2.1 |
| Belgium      | 1.6 |
| Italy        | 1.5 |
| Canada       | 1.4 |
| Spain        | 0.5 |

Figures are for latest year available from OECD. In some cases figures represent research and development as a per cent of gross domestic product (GDP) not gross national product (GNP).

mission-oriented ministries on research and development. But to many academics, this money often seems somewhat out of proportion with the quality of the work being done.

Although it is tempting to suggest that federal research money be shifted from the federal laboratories to universities, Arthur May, president of the Natural Sciences and Engineering Research Council (NSERC) says reducing support to the government laboratories would be a

## Acknowledgements and currency

EXCEPT as noted, this survey of science in Canada was written by Joseph Palca and Alun Anderson.

Our travels took us to Ottawa, Toronto, Edmonton, Vancouver, Inuvik, Montreal, Quebec City, St John's, as well as short stops in between. We are well aware that for every laboratory, office and facility that we visited, there were dozens that we did not, omissions that were forced more by logistics than choice. We hope that what follows represents a fair sample.

Space does not allow us to thank all the people who helped make covering Canada a more manageable task. Special thanks must go to Victor Bradley of the Canadian Embassy in Washington, Yvonne Lenz of the University of Toronto, Jim Guthrie of BeauDril Ltd, Mario Filion at McGill University, Guy Poirier of Université de Laval, and Michael Anderson of St John's University.

All dollar figures refer to Canadian dollars. At present, £1 = Cdn \$2.18, and US\$1 = Cdn\$1.22.

mistake. May argues this is not the time to take money away from any sector performing research in Canada, but instead to find more money to support areas that are in need.

May is encouraged by the recent announcement that the research councils will get an extra \$200 million over the next five years — not a substantial amount, but hopefully at least it will be a harbinger of other changes in federal policy. Another promising sign has been the small but steady increase since 1981 of spending by industry on research and development.

A more controversial suggestion for the government laboratories comes from Stuart Smith, former chairman of the Science Council of Canada. He believes that these laboratories contain an untapped potential for economic prosperity, and he has formed a small company to try to exploit it.

Smith argues that because of government rules, there is virtually no incentive for government scientists to seek com-

#### Research and development scientists and engineers per 10,000 in labour force

|              |      |
|--------------|------|
| US           | 69.0 |
| Japan        | 63.2 |
| West Germany | 49.1 |
| France       | 41.2 |
| Sweden       | 39.0 |
| Switzerland  | 37.0 |
| UK           | 32.8 |
| Canada       | 30.0 |
| Italy        | 27.0 |
| Spain        | 10.0 |

mercial exploitation for their work. To change that, Smith proposes that his company will act as a go between, operating the government-owned laboratories with an eye toward entrepreneurial development. Instead of licensing patents, the government would be given an equity interest in any spin-off companies that might arise, a route that should bring a higher return in the long run.

The climate may well be right for such a change. Certainly the highest levels of government have begun to look for ways to rejuvenate the national laboratories, and younger scientists are sure to welcome an opportunity for extra cash. Those likely to be less happy, however, are the mid-level bureaucrats, who may view any such change as an indictment of their work in the past.

### Universities and industry

The government is looking hard at ways to fill in the missing research and development infrastructure that would normally be provided by industry, and a favoured area for attention is industry-university relations. Governments, both federal and provincial, have tended to view university researchers as 'layabouts', with long summer holidays and a penchant for studying obscure, irrelevant problems.

## Science ministries and superministries

FRANK Oberle is in charge at the Ministry of State for Science and Technology (MOSST). Curiously, this gives him the official title of "Minister of State (Science and Technology)" rather than that of "Minister of State for Science and Technology". The latter title goes to Robert de Cotret who heads the powerful Regional Industrial Expansion ministry and has separate responsibilities for science and technology. To make matters more confusing, both ministries are soon to be reorganized to produce a "new, flagship super ministry" of Industry, Science and Technology (DIST) in which the responsibilities for science and technology will be separated.

Federal science policy is in ferment. As Oberle explains, the government began developing a national science and technology policy back in 1984. They found that "little planning had gone on, compared with other countries . . . industry was doing little research, and the universities and government laboratories were working in isolation". Advice was sought across the country. In June 1986 a conference at Winnipeg announced the formation of a Council of Federal, Provincial and Territorial Ministers of Science and Technology. By March 1987 they had come up with Canada's first National Science and Technology Policy, which exhorted all the different players in science and technology to talk to one another and forge a consensus.

Opportunity for that came in January 1988 when the Prime Minister chaired a three-day "National Conference on Technology and Innovation" for two hundred "key decision makers from the universities and industry" in Toronto. Widely regarded as a huge success, the conference was chaired by the Prime Minister himself, who attended sessions throughout the three-day conference. Playing a pivotal role was the National Advisory Board on Science and

Technology (NABST), a wise-men's council the Prime Minister had set up the previous February.

The result was a commitment from the government to spend an extra \$1.3 thousand million on science and technology over five years. Some of the money was earmarked for a federal centres of excellence scheme (fixed at \$240 million in May), some for scholarships (\$80 million over five years, announced in March), and some



*Oberle: one of a brace of ministers.*

eventually went to the research grants councils (\$200 million over five years, announced in May) and to the space programme (\$184 million, announced in May). The remainder will be looked after by DIST.

When the new ministry is in place MSST will become a subsection within it, headed by Oberle as junior minister. It will act, says Oberle, "as an advocate for the universities and have an auditing function". It will also run the selection process for the new Network of Centres of Excellence, but its planning and coordinating functions for industry, trade, technology, space and communications will go elsewhere within DIST. NSERC and NRC will continue to report to Oberle in the first instance. □

Scientists themselves have probably encouraged this notion by referring to basic or undirected research as 'curiosity research', a particularly infelicitous term. In an effort to get scientists in universities to 'do something useful', there has been a push for technology transfer, encouraging university researchers to take their work to industrial developers.

George Connell, president of the University of Toronto sees nothing fundamentally wrong with this approach. He says there is a large untapped potential for industry-university collaboration.

Connell reckons such collaborations pose little threat to universities, and may have the desired benefit of making industry more aware of the benefits of research, directed or otherwise.

But Arnold Naimark, president of the University of Manitoba, feels such collaborations reflect a fundamental misunder-

standing of the research mission of universities. He says their job is to identify, recruit, and then make long-term commitment to scientists interested in basic phenomena with no application to commercial interest. If that role is subverted by an attempt to focus university research on 'relevant' problems, the necessary cadre of basic research scientists and teachers will be lost to future generations.

The academic community feels particularly abandoned by the Conservative government of Brian Mulroney, because one of his campaign pledges was to raise Canadian spending on research and development from 1.4 per cent to 2.5 per cent, bringing it more in line with other industrialized countries. But soon after the elections, that promise seemed to be ignored. Instead of a massive influx of cash for science, the government responded to pleas from the granting



councils for more money with the matching funds scheme.

## Matching dollars

Ballyhooed as a plan to raise the councils' spending authority by \$1,000 million between 1987 and 1992, the matching fund scheme in fact ties spending on basic research to investment by the private sector. As shown in the table (page 722), the government leveled the base budgets of the granting councils — the National Science and Engineering Research Council the Medical Research Council (MRC), and Social Sciences and Humanities Research Council (SSHRC) — so that any increased money is tied to corporate contributions to university research.

Under the scheme, the government contributes one dollar for every eligible dollar contributed by the private sector, subject to annual ceilings, with increases at approximately 5 per cent per year.

Although there was great trepidation about the scheme when it was first announced in 1986 (see *Nature* 320,102; 1986), most agree that it has led to growth in funding for research, even though the rate of increase is still scarcely a percentage point above inflation and well below inflation in research costs.

But university researchers still see the scheme as a flawed attempt to satisfy two separate demands. The grants councils wanted more money for basic research, without strings attached.

The government wanted to use the grants councils to help stimulate research in industry. The result was a scheme in which the grants councils budgets were tied to increased investment in university research by industry, but not tied in any meaningful manner. The MRC simply records eligible investments by industry, which have always been more than sufficient to trigger the maximum possible contribution in federal matching funds, and gives out the money for peer-reviewed basic research. "Just an accounting function which allows the government to abrogate its responsibility to support university research" is how Lewis Slotin, Director of Programs at MRC describes the scheme. "If I wanted to stimulate industrial research, I'd earmark money to deliver through university-industry collaboration", he says. Indeed, MRC and NSERC already have such schemes (see page 722).

NSERC administers the matching funds scheme a little differently. Not all the matching fund money goes in a common pot; some is used to reward universities that raise money from industry (now 10 per cent, rising in two years to 30 per cent of the amount raised). Critics cry 'kick-backs' and 'porkbarrel' as they see matching-fund grants given out by a criterion departing from peer-reviewed scientific excellence. Smaller universities, with little chance to raise money from industry, pro-

## The hazardous business of being critical

THE Science Council of Canada operates 'at arms-length' from the government, conducting evaluations of Canada's scientific institutions. But it is in the unfortunate position of offering advice without having any authority to make people listen, and reports that criticize are rarely welcome. The current government went so far as to cut the council's budget in half. But with an enthusiastic and energetic new chairman, there may be better times ahead.

The council now operates with a staff of about 30 people and a budget of \$2.8 million. The new chairman, Geraldine Kenney-Wallace, is from the University of Toronto where she is a member of the laser research group. But Kenney-Wallace appears to have access to the government's inner circles, serving on virtually every national committee addressing science policy issues, including the National Advisory Board on Science and Technology and the Premier's Council in Ontario.

Kenney-Wallace says she has been able to wield some influence within government because she is apolitical. Her predecessor, Stuart Smith, was an active player in the Ontario Liberal party. Kenney-Wallace is also a winner of the NSERC Steacie award, given to the country's best young scientists. She says if she finds the government is shutting her out of policy decisions, she can always return to full-time work in her laboratory.

The Science Council's most recent report examines the potential of university-industry collaboration (*Winning in the World Economy*). Another report has dealt with the opportunities for Canada's biotechnology industry (*Seeds of Renewal*), and earlier this month the Council released an analysis of the sustainable use of water through to early the next century (*Water 2020*), of particular interest to Canada with a fifth of the world's fresh water reserves. □

test as they see themselves cut off from the action. Even bigger universities admit that the scheme has not actually stimulated industry to invest more.

## Equitable treatment

Ironically, both NSERC and MRC are criticized for being fair. Although one quarter of the NSERC's operating funds go to the top 10 per cent of grant holders, there is still the perception that NSERC and MRC temper the reward of merit with the recognition that distribution must be equitable. This is in spite of the fact that many selection committees have a strong international membership. An equitable distribution implies that grants must be spread out over the regions, balanced between francophone and anglophone communities, and not allowed to grow so big that others go without.

Some see regionalism as a good thing, allowing researchers to carry at least a bare-bones research programme at remote universities. But because it is easier to keep existing operating grants than to start new ones, there is a tendency for this regional distribution to be self-perpetuating once it has started.

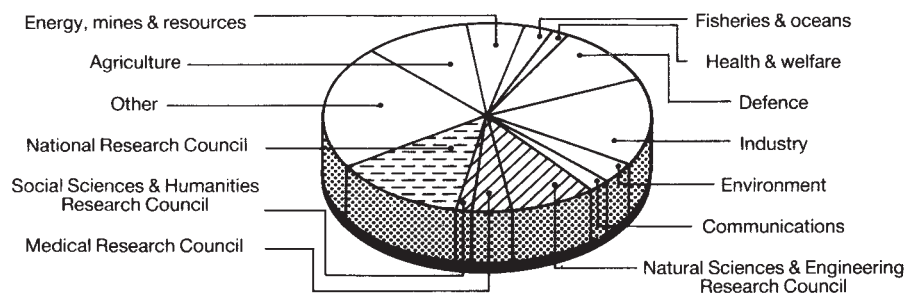
But others feel that equity is a barrier to Canadian research excellence.

preventing top researchers from getting the money to follow through on promising lines of research. Michael Hayden, a medical geneticist from the University of British Columbia, points out that "one of the most frustrating things" for Canadian researchers is "that it is easy to get started but hard to make a group grow beyond a small number of researchers".

NSERC is aware of the problem and is considering a strategy of "significant increases" to the top ten per cent of researchers. But many scientists point out that any further concentration on excellence would be cruel, given the number of good grant applications that already have to be turned down.

The most obvious — and perhaps least likely — solution to this dilemma is for the grants councils to receive a large infusion of new money. Five-year plans prepared by the Council have consistently called for a doubling of its budget and have been backed by the Prime Minister's advisory board.

By some arguments, Canada is already spending enough on research. Fraser Mustard, head of the Canadian Institute for Advanced Research, says it is possible to use the rule-of-ten to make comparisons with the United States. US National



How the cake is divided — the recipients of the total government research spending.

## Slicing up the research cake

■ WITH the exception of medical research, all federal support for the natural sciences and engineering comes from the **Natural Sciences and Engineering Research Council**. NSERC consists of a 22 member governing council, two thirds from academic institutions, the remaining third from industry. Grants are made by the president of NSERC, currently Arthur May, on advice from some 40 advisory, *ad hoc* and selection panels.

NSERC spends approximately 60 per cent of its budget on its research base; operating grants to individual researchers, grants for academic collaborative research and grants for equipment and facilities. The next largest piece of the NSERC pie goes for grants for targeted research (17 per cent), followed by scholarships and fellowships (16 per cent). The former category includes university-industry collaborations that are jointly financed by NSERC and interested companies, and strategic grants for projects "in certain areas of national concern." This phrase can refer to anything from biotechnology to robotics, but the projects tend to be ones where a commercial payoff can be readily imagined.

The remaining 7 per cent is split between NSERC operating costs and general research support.

There is no question that NSERC has more researchers to support than it has research dollars to support them. In the 1986-87 fiscal year NSERC made some 6,100 operating grants, but the average size as was only \$23,000. Although operating grants go entirely to the direct cost of research, with no money deducted for indirect costs incurred by the host university, these numbers are small by inter-

national standards.

NSERC still sees its primary role providing adequate support for the foundation of the research pyramid — the university scientist — NSERC sees an increasing role increasingly in support of research programmes that are intended to have a more direct economic impact. This will also mean spending more to bring new people into the science and technology pipeline.

As always, this strategy requires money not currently available. By the 1990-91 fiscal year NSERC's plans require \$622 million, but currently approved funds, assuming full matching from industry, amount to only \$404 million.

■ UNLIKE the British body of the same name, the **Medical Research Council** has no laboratories of its own and gives grants only to cover the direct costs of biomedical research. It is by far the biggest player in the field: this year, it will provide \$180 million, a little more than the total of \$176 million coming from all provincial, non-profit and foreign funding bodies.

MRC's policy is simply to back the best, its few experiences with targeting particular areas for development having been less than successful. Last year its 750 new grant applications were examined by 2,500 referees, around a third of them scientists in the United States and other foreign countries. Twenty-two advisory panels represent the different areas of biomedical research.

MRC is often the only body to which

certain biomedical researchers can turn. Neuroscientists, for example, rely on MRC for 85 per cent of all their research funds. A grant refusal is thus in all practical purposes a kiss of death for a particular project. Cardiovascular and cancer researchers have more choice and can, on average, look to a variety of charitable, regional and scientific society-linked foundations to provide between half and two-thirds of their costs.

Most of MRC's money is spent in hospitals attached to universities rather than in universities themselves. That is because over the years hospitals have proved more flexible than universities in finding ways to obtain the infrastructure for big new facilities and have had staff who can commit themselves fulltime to research. They have also been skillful at raising private money through foundations and appeals.

The most prestigious of MRC's grants

Matching funds (\$ million)

|                  | 1987  | 1888  | 1989  | 1990  |
|------------------|-------|-------|-------|-------|
| NSERC            |       |       |       |       |
| Base budget      | 312.6 | 313.6 | 313.6 | 313.6 |
| Matching funds   | 25.4  | 40.5  | 64.0  | 90.4  |
| Total with match | 338.0 | 354.1 | 377.6 | 404.0 |
| MRC              |       |       |       |       |
| Base budget      | 161.6 | 161.4 | 161.4 | 161.4 |
| Matching funds   | 10.1  | 20.9  | 33.2  | 46.8  |
| Total with match | 171.7 | 182.3 | 194.6 | 208.2 |

are its group grants, worth \$1-2 million per year, and the salary support grants given to a handful of distinguished investigators. In total, 66 per cent of total funding goes to grants-in-aid of research, 15 per cent on group and programme grants, 6 per cent on salary support, and 10 per cent on training. One per cent is used to stimulate directly industry-university collaborations. □

Science Foundation budget is about US\$1,700 million, so by Mustard's argument NSERC's budget of \$354 million compares favourably. But the difference is that for Canada, NSERC is the only game in town for the natural sciences, whereas in the United States researchers may turn to the Departments of Defense, Energy, Agriculture, Commerce and the National Aeronautics and Space Administration for support, not to mention the university based programmes conducted by many large research-based companies.

### Centres of excellence

On May 25 the government at long last announced that it would spend \$240 million on 'Networks of Centres of Excellence'.

The scheme could be the best thing that has happened to Canadian science in years — or it could sink into mediocrity. The key factor is whether scientific excellence will triumph over politics in deciding what centres should be established and where.

The scheme is a blatant imitation of Ontario's Centre of Excellence scheme. It aims to build links between the universities, industry and government by collaborative research centres chosen for their excellence, potential for commercial result and ability to establish networks among researchers scattered over the country. As many as twelve centres may be funded. Competition for them is likely to be intense. The promise of the first new money for years is stimulating scores of groups to rush together proposals.

When Ontario Premier David Peterson launched his Centres of Excellence scheme (see page 728) he told his advisors not to worry about where the money went within Ontario but to pick the best, even though he might be criticised for 'elitism'. Will the federal government be as brave in the face of loud demands from the provinces for 'equitable treatment'? Cynics have been predicting that the government will yield to pressure and place one centre in each of main geogra-

phical regions.

Round one has gone against the cynics. According to the May announcement, proposals for centres are to be assessed by peer review organised by the grants councils. Implementation of the scheme will be supervised by Gilles Cloutier, Rector of the Université de Montréal and John Evans, Chief Executive Officer of the biotechnology company Allelix. They will report to the Minister of State (Science and Technology) Frank Oberle who will report to the Cabinet. There political pressure may be brought to bear. But the government has said that the ranking of proposals for centres of excellence will be made public after a decision has been made (as was done in Ontario). That should expose any political deals.

Requests for proposals are due out in a matter of weeks, and the proposals will be due back by the end of the summer. The governments choices should be revealed before the end of the year.

In the rush to get proposals in for the



new scheme, scientists may forget why it is that it bears the unwieldy title of 'Networks of Centre of Excellence'. The need for 'networks' has not actually been dictated by scientists, who are usually adept at linking themselves into international networks (more often called grapevines). Networks have become a political expedient, designed to make it easier to avoid trouble with the provinces by smearing the new centres over the whole of Canada, wherever their headquarters might be. But as John Polanyi, the University of Toronto Nobel Laureate points out, "if you want to get something out of science, you have to respect the internal logic of science. . . . stylistic guidelines from the government are not going to help, in fact they are going to hinder".

## Bill C-22

A second boost for Canadian science is on the way from the pharmaceutical industry. In exchange for a new patent protection law, Bill C-22, the industry has promised to spend \$1,400 thousand million dollars extra on research and development in Canada over the next eight years.

Pharmaceutical research in Canada slowed to a crawl in 1969 when an amendment to the Patent Act permitted companies to copy new drugs just four years after patenting, instead of the twenty years usual in other countries. The result

### Federal research and development income

| <i>Institution</i>             | <i>\$million</i> | <i>Share</i> |
|--------------------------------|------------------|--------------|
| University of Toronto          | 63.3             | 12.3%        |
| McGill University              | 44.0             | 8.6%         |
| University of British Columbia | 37.3             | 7.3%         |
| McMaster University            | 25.1             | 4.9%         |
| Alberta University             | 24.4             | 4.8%         |
| Université de Montreal         | 24.0             | 4.7%         |
| University of Western Ontario  | 23.2             | 4.5%         |
| University of Manitoba         | 22.0             | 4.3%         |
| Waterloo University            | 21.8             | 4.2%         |
| Laval University               | 20.5             | 4.0%         |
| Total for 80 universities      | 513.1            |              |

The top ten universities account for 59.5 per cent of all federal spending on research at universities.

was a rapid growth in the generic drugs market industry and, consumer associations say, a fall in the cost of drugs.

But big drug companies, all of them multi-national companies capable of switching operations around the world, stopped investing in Canada. They claimed that they could not hope to gain a fair return on their enormous research costs if generic drug makers were allowed to manufacture copies of new drugs so quickly.

Despite opposition from consumer groups and the generic drug industry, the government compromised in December 1987 by passing Bill C-22 which provides seven to ten years of exclusive marketing

# Recovering from budget setbacks

THE National Research Council (NRC) is still Canada's largest and most diverse research organization. But it is not the power in the land that it once was. Its responsibility for university research grants was removed in 1977 and given to three new granting councils. And since then it has had to absorb a disproportionate share of government cuts.

NRC officials see these changes as evidence of the organization's success. After the Second World War they were charged with building up Canada's science and technology capabilities. Once they had succeeded they found the newly created research community wanted greater independence from NRC and its chain of large laboratories.

Over the past few years lack of funds has forced it to shut down several facilities, including the Algonquin Radio Observatory and the Fort Churchill, Manitoba rocket launch facility, and to abandon plans for the Cold Regions Laboratory in Alberta. Numerous programmes have been reduced. Despite this, NRC still receives the largest single share of federal science and technology dollars, and has established priority areas that will grow even in the constrained financial climate.

NRC is a crown corporation, reporting to Parliament through the minister of state (science and technology). A 21-member council governs NRC, with membership drawn heavily from industry, with a sprinkling of academic scientists. Its budget is currently at \$450 million, a modest increase over 1987. Some two-thirds of that amount is spent in the Council's own research laboratories.

NRC's worst budget blow came in 1984, according to Ross Pottie, NRC executive vice-president when the new government asked for \$70 million in cuts with two weeks' advance notice. At that time there were numerous layoffs, several programme closures, and accusations that the cuts were being made wantonly, without regard to scientific merit. A tribunal appointed by the science minister ultimately determined

that the cuts were made appropriately.

A second financial assault occurred in 1986 when the government pared NRC's budget by a further \$20 million. NRC cut approximately a dozen programmes, cancelled plans to refurbish the Algonquin Radio Observatory, cut the budget at the Dominion Astrophysical Observatory in Victoria and reduced support for TRIUMF, the Canadian meson facility in Vancouver (see page 725).

NRC did successfully negotiate with the United Kingdom for a 25 per cent share in the James Clerk Maxwell Telescope in Hawaii.

Biotechnology has been given a favoured place in NRC's long-range plans. In addition to research in Ottawa, NRC also liberally supports a large new Biotechnology Research Institute in Montreal (see page 731), and the Plant Biotechnology Institute in Saskatoon.

Space science has also received special treatment from NRC. The government's commitment to the US Space Station means an even greater percentage of NRC funds will go to space related activities, especially as the space station moves into its construction phase. This large commitment to manned spaceflight, reckoned to cost Canada \$1,200 million, has angered many in the scientific community who see vast sums going to a narrow area of research.

NRC also runs a unique scheme designed to get the fruits of research out of the laboratory and into industry. Throughout the country, Industrial Research Assistance Program (IRAP) employees provide a free, personal and confidential "family doctor"-style service to industry, paying on-site visits to smaller Canadian companies that lack technical expertise. They, in turn, can refer back to NRC's technical information service to try to solve specific technical problems. The scheme seems to be working: analysis of IRAP's 4,000-5,000 clients a year show that adding technology allows them to grow faster than their competitors. □

rights to drug patent holders.

In exchange the pharmaceutical industry promised to boost research spending from 4.9 per cent of sales to 8 per cent in 1991 and 10 per cent in 1996. That should produce an extra \$1,400 million in research and development expenditure on top of the \$1,600 million the industry had already planned to spend by 1992.

Around a third of the money should find its way into the universities, where at first it is likely to be spent on clinical trials. Later, investments in new facilities and research centres at universities are expected.

Critics point out that there is no guarantee that the money really will be spent — industry's promise is only a promise and

not a legally-enforceable commitment.

But enough university and hospital laboratories already report approaches from big companies (and from head-hunters seeking to staff expanding company laboratories) to dispel cynicism.

The federal government's efforts to give pharmaceutical research a rousing kick-off have back-fired. The government decided to transfer \$100 million over ten years to the provinces to spend on research. The first \$25 million (divided up according to population) has already gone out. But the government forgot to tell the provinces what the money was for, and some of it has simply vanished into provincial general accounts. □

# Organization and reorganization preoccupy Canada's scientists

THROUGH the kindness of George Connell, the president of the University of Toronto, *Nature* was able to hold a consultation with officials, academics and working scientists at an afternoon-long seminar on the university campus. Much of the conversation informs the accompanying pages; what follows here is a brief account of the issues that appear to be at the front of the research community's mind. Attending were Maurice L'Abbé (Science and Technology Council of Quebec), George Connell (University of Toronto), Michael R. Hayden (University of British Columbia), Tom Jukes (University of California), Geraldine Kenney-Wallace (Science Council of Canada), Gordon MacLachlan (McGill University), Arthur May (Natural Sciences and Engineering Research Council), Arnold Naimark (University of Manitoba), John Polanyi (University of Toronto), Mark J. Poznansky (University of Alberta), Scott Tremaine (Canadian Institute for Theoretical Astrophysics), J. Tuzo Wilson (University of Toronto) and from *Nature*, John Maddox (Editor), Alun Anderson (Washington Editor) and Joseph Palca (US News Editor).

In the old days, Canadian science was almost synonymous with the National Research Council (NRC). Tuzo Wilson, the geophysicist, explained why that should have been the case; NRC became the residual legatee of the great excitement of Canada's contribution to the Second World War, as a safe base both for general defence research within the British Commonwealth and, before Pearl Harbor, for the British contribution to the eventually joint development of nuclear weapons.

The momentum was sustained, after 1945, with the development of the CANDU reactor, the continuing influence of the Defence Research Board, and the strength



John Polanyi.

of G.A. Herzberg's spectroscopy research laboratory at Ottawa, in all of which NRC had an influential hand. Some of NRC's riches trickled as research grants to university groups, but like crumbs from rich men's tables. In less than a quarter of a century, NRC had become an over-powerful paternalistic Goliath, puzzled that its good intentions were so often misunderstood, until a Trudeau government cut it down to size.

Canada has been searching for a comfortable organization ever since. Events have not been conducive to success. Reorganization has coincided with the growth of regional self-consciousness, which explains why some insist on the importance of networks among academic researchers as a means of demonstrating that inter-provincial collaboration is a reality and why others say flatly that networks are a sham, a device for winning a cosmetic benefit at the cost of efficiency and the concentration of inadequate resources on outstanding research groups.

The shortage of money, every research community's first complaint, takes a distinctive form in Canada. What funds there are for academic research are over-equitably spread, with the result that researchers find themselves supported with grants that are perhaps a sixth of what their competitors south of the border are awarded by agencies such as the US National Institutes of Health (NIH). Yet the device of applying directly for NIH support often requires a demeaning deal with a US research group.

Quite apart from the political pressure in favour of inter-provincial equality, there seems no general agreement on the principles on which resources might be concentrated. The federal government and its advisers favour concentration on national needs — energy, resource development and the like. But some would rather concentrate on fields in which Canadian researchers have made an international mark (such as recent successes in the identification of genes responsible for inherited diseases) and others (such as John Polanyi) would concentrate instead

on outstanding people. Yet the support mechanisms, especially those by which federal dollars match what researchers are able to recruit from industry, imply that academics often resent the way their work is biased by industry's conception of its own needs.

What are the chances that these discontents will melt away under the influence of enlightened policies yet to be enacted? One surprising undertone of this consultation was what seemed to be a general sympathy for the federal government's predicament. The shortage of funds seems to be generally appreciated, as is the difficulty of working out a strategy for the years ahead. Some of the thrashing around of the past two decades would have been more severely criticized than it was at *Nature's* seminar if people were more inclined to believe the federal government to be malign rather than lost.

It may have helped to blunt the edge of criticism that the newly appointed chairman of the Science Council of Canada, Dr Geraldine Kenney-Wallace, is a regular academic (from Toronto) who, however



Arthur May (top) and Michael R. Hayden.

controversial her business may be, retains a kind of academic articulateness and independence. Indeed, one could almost hear the Canadian participants in our consultation catch their breath when she gave us to understand that if she found the bureaucracy cramping her freedom of action, she would simply quit, going back to being a simple chemist. If she can win the trust of her natural constituents, Canada's grant-holders and academics, she may find that she never has to take that fateful step.

John Maddox



J. Tuzo Wilson.



# British Columbia on the lookout for its place in the sunshine

## Vancouver

BRITISH Columbia has some of the same reputation in Canada as California does in the United States. To eastern Canadians, British Columbia is the land of the lotus eaters, a province of great natural beauty, relatively mild climate, and great wealth from natural resources.

Logging has been British Columbia's big industry. Forests cover 55 per cent of the province, and there are 47.8 million hectares of commercial forests. But as old-growth forests diminish and what remains becomes more remote, industry is realizing that the days of plenty have limits. Company annual reports have begun to talk about value-added products as a way of keeping future prospects rosy.

But the move away from reliance on natural resources for prosperity is slow, even for forward thinking companies.

MacMillan Bloedel Ltd, the largest forest products company on the West Coast, had sales of more than \$3,100 million in 1987. But its research and development budget was only \$20 million, and Otto Forgacs, vice-president for research

says the emphasis is on development. He says that it is growing increasingly easier to get corporate backing for research projects, a job that will be made still easier if sales of a new wood composite product that MacMillan Bloedel developed called Parallam can live up to its technical promise.

British Columbia's research enterprise got a tremendous boost earlier in this decade from a stirring journey taken by a young man with cancer. Terry Fox had his right leg amputated to prevent the spread of cancer. In 1979 he determined to run across the country to raise money for cancer research, and died in the attempt, but not before attracting international attention and raising some \$35 million. His effort had a tremendous effect on the country, and in his native British Columbia there are numerous tributes to his memory, including a Mount Terry Fox in the Rocky Mountains. The provincial government in 1981 formed the Terry Fox Foundation, a charity that is already having an impact on biomedical research in the province (see page 726). □

Natural Sciences and Engineering Research Council (NSERC), the affiliated universities and Atomic Energy of Canada Ltd. It is located on the University of British Columbia campus.

Vogt says that TRIUMF has a unique role to play in understanding subatomic physics. By studying the decay products of W and Z particles first discovered at CERN in 1983, researchers at TRIUMF were able to confirm the left-handed asymmetry of these particles. A facility like TRIUMF is needed to produce a sufficient muon flux to conduct such studies. TRIUMF's high muon flux has also been useful for materials science studies, notably in determining the atomic structure of the new class of high critical temperature ceramic superconductors.

Pions produced at TRIUMF are also being used therapeutically. Vogt is particularly proud of a patient with an inoperable glial blastoma whose tumour has been effectively removed with pion radiation.

Canadian scientists are divided over the kaon factory. Non-physicists are naturally worried that the estimated \$571 million project will divert funds from other areas of science. Even among physicists there is some question over whether a kaon factory is the best way to spend limited funds.

Vogt has received a \$100 million commitment from the province of British Columbia, and that should provide some leverage with Ottawa. But the kaon factory is competing with other big projects, such as Radarsat and the National Centre for Ocean Studies in St John's, Newfoundland. Even with international support covering one third of the cost, the kaon factory will need all of Vogt's infectious enthusiasm if it is to receive financial backing from the federal government. □

# Seeking back-to-back TRIUMFs

## Vancouver

ERICH Vogt's enthusiasm for subatomic physics is infectious.

As director of TRIUMF, one of only three large meson factories in the world, Vogt had to fight a debilitating 14 per cent cut in funding by the National Research Council (NRC), eventually convincing a tight-fisted federal government that all the money should be restored. Now he is pushing hard for a major upgrade of TRIUMF to create a 'kaon factory' capable of operating at energies up to 30 GeV. Vogt is constantly transmitting his enthusiasm, hoping to produce suitable infections that will yield dollars for the new facility.

Plans for TRIUMF were approved in 1968, and the first full energy, 520 MeV beam was achieved a decade later. The 18-metre-diameter cyclotron has six radial arm magnets, with radio frequency cavities accelerating negatively charged hydrogen ions.

To produce mesons and pions, a stripping foil is placed in the beam to remove the two electrons from each hydrogen ion, allowing the protons to bend out of the accelerator. Three proton beams emerge from the cyclotron, dividing into a variety of experimental chambers.

The proposed kaon factory will use the existing cyclotron as an injector for a 3-GeV booster ring, the output of which will enter a 30 GeV synchrotron. The high-

energy protons will be directed at targets to produce kaons, antiprotons and neutrinos.

Despite its name — the Tri-University Meson Facility — TRIUMF is managed by four universities; the University of British Columbia, the University of Victoria, Simon Fraser University and the University of Alberta. The NRC provides 80 per cent of TRIUMF's \$32 million budget, most of the rest coming from the



TRIUMF weighs in at over 4,000 tonnes, with a magnet 18 metres in diameter.

## High technology in space and under water

### Vancouver

Although Canada has traditionally been a resource-based economy, the number of successes by high-technology companies is growing. Two British Columbia companies show the promise and the pitfalls.

MacDonald Dettwiler and Associates Ltd has built an international reputation in aerospace electronics. International Submarine Engineering (ISE) Ltd is one of a small number of companies worldwide producing autonomous submersible vehicles. Both have done well, but both have to look outside Canada for their markets.

MacDonald Dettwiler started nearly 20 years ago in John MacDonald's basement and now has \$65 million worth of business. The company is a leader in digital processing of remote-sensing data. They have shown the feasibility of making 1:50,000 scale topographic maps from stereo images from the French SPOT satellite, having pioneered digital image processing of synthetic aperture radar telemetry from the US Seasat satellite. MacDonald Dettwiler also produces a synthetic aperture radar for light aircraft.

But MacDonald says Canada has not shown steady support for its domestic industries. MacDonald Dettwiler already does 80 per cent of its business abroad and without a commitment for new Canadian space ventures like RADARSAT, Canada's aerospace industry will rely even more heavily on the largesse of foreign countries for sales.

ISE faces similar problems, but on a smaller scale. Having designed Dolphin and ARC, small, cost-efficient autonomous submarines for ocean-floor surveys and monitoring offshore drilling equipment, ISE has had to turn to unlikely markets. Two ISE submarines are currently carrying visitors to Fantasyland at the the West Edmonton Mall, and a new submarine has been built for near-shore tours. At the same time, the Canadian government is considering a multibillion dollar purchase of nuclear-powered submarines to defend the Canadian coastline. ISE President James McFarlane says a fleet of autonomous vehicles could do some of the patrolling tasks at a fraction of the cost.

A reluctance to accept new technology is holding back another area of ISE's business. McFarlane says his autonomous vehicles can do complex tasks on drilling rigs, often replacing human divers. The technology exists, but the corporate mindset does not. McFarlane hopes that with time, that will change. □

## Putting an old model in a new place

### Vancouver

THE Terry Fox Foundation wants to repeat a success story.

Using the model of the Wellcome Foundation, it has created the Biomedical Research Centre (BRC) to conduct basic biomedical research, as well as Pacific Pharmaceuticals, a company that the foundation hopes will someday sell the fruits of BRC research. If it works, the foundation will have a self-perpetuating source of funding. The Wellcome Foundation is sufficiently convinced to put up half the capital to start BRC.

For the present, operations are just getting under way. Last month, BRC officially opened its new research building on the campus of the University of British Columbia. John Schrader, former head of the immunoregulation laboratory at the Walter and Eliza Hall Institute of Medical Research in Melbourne, Australia, is BRC's director. He has a budget of approximately \$2.5 million per year for five years to get BRC off the ground, but

additional funds will be sought from Canada's granting councils to enable BRC to keep going.

Work at the centre will focus on signal transduction through receptor/polypeptide interactions. One project that the centre expects to get started on soon is improving automated peptide synthesis. Other work will focus on serine and threonine kinases, leukaemogenesis and polypeptide hormones. A tunnel connects the new BRC building to the university hospital next door, and Schrader expects clinical work will begin soon.

The key to making BRC a success is to encourage collaboration among independent scientists, according to Schrader. The new facility has few walls, making it difficult for researchers to hide away in a corner to conduct experiments.

"If you want to make a mark while working outside the big centres of science, you've got to get people from different disciplines pooling their resources", Schrader says. □

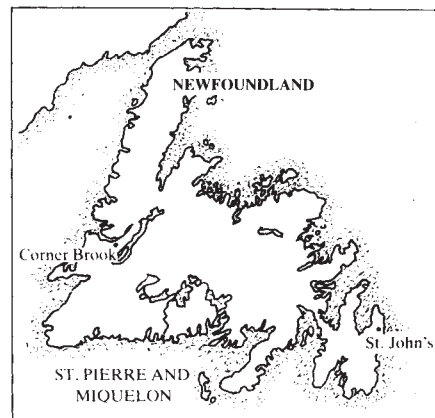
## Newfoundland thrives on cold water despite cod wars

### St John's

St John's Newfoundland was the first part of North America to be settled — by Vikings around AD1000 — but the last province to join the federation. Even now Newfoundlanders debate whether they made a mistake in 1949 and would have been better off independent. Newfoundland is not too small for survival; the province, which includes mainland Labrador, is one and a half times the size of Great Britain. But most of that land is Labrador which has only a tiny part of the province's population of 600,000.

For much of the sixteenth century, Newfoundland was at the far edge of the known world; a place of refuge for fisherman who sailed from Europe to the rich, shallow waters of the Grand Banks. French and English fought over the rights to fish, with the port of St John's being captured and recaptured. The Treaty of Utrecht set the present-day boundaries in 1713 and, unintentionally, ensured that the "cod war" would still be running in the twentieth century. The Treaty gave Newfoundland Island to England but left France with the tiny islands of St Pierre and the Miquelons, just a few miles from the coast.

After Canada claimed a 200-mile economic zone around its coasts in 1977, France did the same around its islands, creating a large contested zone in which France will not obey Canadian fishing quotas. Canada has accepted only a twelve-mile limit around the islands, and



there is no sign of the cod war ending. Both countries are avoiding the use of force, but Canada has intercepted and arrested ships flagrantly violating its territorial limits — including one boat defiantly decorated with tricolours.

The fisheries are still the island's biggest source of revenue, although on the mainland they are far outdone by the gigantic iron ore mines at Labrador City. The paper and pulp industry comes third in importance.

With the emphasis on looking after a scattered population, many of them involved in the fishing industry, the provincial government has had little interest in science. But that is changing. A science council to advise the Premier has just been set up, with Memorial University of Newfoundland's Dean of Science, Louis Visentin, as chairman. □



# Memorial University using oceans to get on the map

## St John's

THE Memorial University of Newfoundland at St John's may be a long way from anywhere but that does not stop it having dreams of greatness. Last month it launched its "Oceans 2000" programme designed to bring almost a billion dollars of investment to the university over ten years and transform it into a world centre in ocean science, engineering and technology.

The first step in the programme is to establish a National Centre for Ocean Studies. Pushing the plan is geologist David Strong, the university's academic vice-president. He plans to seek \$283 million in federal funding, and Strong is bullish. "We're going after it across the board and we're going to win," he says. The rest of the money will, with luck, come from the research councils, regional sources and development agencies.

The project is likely to appeal to the government because of the industrial investment it could attract to a technologically backward region. St John's is nicely placed to exploit the growing economic importance of the cold oceans. It sits right on the Atlantic with easy access to the Arctic.

Offshore, on the Grand Banks is one of the world's largest fisheries. And soon, the go ahead is expected for the development of the Hibernia oil and gas field, with massive investments from the petrochemicals industry.

The university believes it can offer a nucleus of special skills to speed the development of ocean science and technology. The Earth Sciences Department, proclaims Strong, "is the best in the country". It is certainly true that, as Strong says, "Newfoundland has got the rocks"; a large expanse of exposed Appalachians and, in the Avalon peninsula on which St John's stands, North America's only bit of Europe — a part of the Eurasian plate that went the wrong way.

From its terrestrial base, the department has moved into submarine and marine geology. That gives the university part of its claim to become an ocean centre; nearby are the Institute for Marine

Dynamics, the Oceans Science Centre, the Ocean Engineering Research Group and the Centre for Cold Ocean Resources Engineering. But still lacking is a ship. An independent part of the expansion plan is a request for a 60-metre ice-capable all-season research vessel to be ready by 1990.

If built, it will incorporate a submarine robot that can operate under the ice and the ability to receive satellite sea surface imagery in real time.

A key part of the expansion plan is certain to be the Institute for Marine Dynamics, recently built on the campus by



Another element of the Memorial University expansion plan will be the Ocean Sciences Centre, housed at Logy Bay, just outside St John's, in this building modelled on the form of a sea urchin. The fine May weather is deceptive: in winter, pack ice that has drifted from the Arctic along the Labrador current fills the bay. The ice sometimes brings polar bears far south from the Arctic. Even in May the last of the season's massive icebergs were drifting along the horizon.

The centre, with a staff of 45, has a full set of marine laboratories equipped with running sea water. Natural Sciences and Engineering Research Council provides almost \$2 million a year for projects that run from the energetics of planktonic food chains to navigation by harbour seals.

the National Research Council. The institute tests ships and offshore structures, especially those designed for use in arctic seas. Its ice tank — the world's largest at 90m by 30m — is housed in a massive building refrigerated to  $-30^{\circ}\text{C}$  and used to see how surface ice interacts with hull, propellers and rudder as a ship breaks through it.

The institute is already a success, constantly busy with some \$7 million of orders a year from customers from all around the world. It will be much busier when a wave basin is completed in 1990. There, computer-controlled wave makers and fans will make it possible to test ships and offshore structures in complex wave conditions that model the severe storms of arctic waters.

Although ocean sciences is where big growth may come — and competition for federal funds will be tough — that is not all

## Health provision goes online

### St John's

WITH Newfoundland's population thinly spread, and travel slowed by a long broken coastline, Memorial University has used its imagination to provide remote educational and health services. It has come up with the "Telemedicine Centre", run from the faculty of medicine.

The centre does not actually use television, as its name might imply, because trials showed it to be unnecessarily expensive. Rather, it provides an interactive audio network linking a hundred communities, enabling groups to sit down together at conferences, consultations or classes. The network handles 3,500 hours of traffic a year; half of it medical data transfer and continuing health education and half of it university and educational courses and government department administrative meetings.

Now, audio is just beginning to be supplemented with an 'electronic blackboard'. A teacher's sketches, made on a special pad, can be instantaneously transmitted to a screen at the students' locations; the teacher can also call up diagrams from disks stored at each location and add to them, or point to particular features, while talking. By this method the system has been able to add visual aids without the huge costs of high-bandwidth video transmission.

The system has been ingeniously adapted for medical diagnosis. Single images — of an injury or infection, for example — can be sent over the same telephone lines by scanning them electronically and stretching transmission over a 70 second period. Soon, X-ray images, as well as EEG/ECG data, will be sent in the same way.

Although established for Newfoundland, the system can reach anywhere there is a telephone. A link to the Universities of Makerere and Nairobi in Africa has already been tested and seems set to provide a cheap way to export medical expertise. □

the university is. Elsewhere, there are numerous small groups that have developed the capacity to do sophisticated research in a university that, twenty years ago, had ambitions restricted to teaching.

More than \$9 million a year in research grants comes to the 250 staff in the faculty of science, an amount per head not far behind the big universities. These researchers are also looking out for new opportunities. The biochemistry and medical researchers are eyeing the money returned to the province as a result of changes in drug patent law (see p.723) and hoping the Premier, and his new science council, will direct the money to basic biomedical research. □

# The shot heard across Canada: Ontario's warning is heeded

## Toronto

In all of Canada, one scheme stands above all others as a sign that politicians can be persuaded to back academic science. The Ontario Centres of Excellence programme, backed by \$204 million in provincial funds, has set an example of a new approach to government support for research. Whether or not the scheme works, it has had the effect of a warning shot across the bow for federal spending on research, putting the federal government on notice that the provinces are willing to do more than talk about the decline in spending for research and development. A national centres of excellence scheme has recently been announced (see p. 722), and the similarities to Ontario's plans can hardly be missed.

Ontario's young, Liberal Premier David Peterson put his political muscle behind the Centres of Excellence programme. He is untroubled by the charge that the federal government has stolen the Ontario plan. "They're copying us, and I don't mind that. It is the sincerest form of flattery", says Peterson. "But, are they copying us well?"

The centres programme is just one of a series of initiatives for which the provincial government intends to spend \$1,000 million over the next 10 years. They all spring from a unique body in Ontario known as the Premier's Council. Chaired by Peterson, the council is made up of influential industrialists and academics who sit outside the normal ministerial organization of the government. Peterson says his only instruction to members is that they "check their special interests at the door", concentrating on what is good for Ontario.

There are three primary components to Ontario's plans. In addition to the centres of excellence, there is also a university research incentive fund to encourage university-industry collaborations with matching funds for eligible collaborations, and the industry research programme to stimulate cooperative research and development projects among companies. Ontario is the wealthiest and largest (by population) of Canada's provinces. But as a recent report from the Premier's Council makes clear\*, the province's future economic health will require moving towards value-added manufacturing for exports, and away from dependence on basic commodities like sheet steel or newsprint.

There are currently seven centres of excellence programmes, chosen from 28 submissions. They are intended to stimulate research, train young researchers, and help deliver technology developed at universities into the private sector. Eight

Ontario universities are involved in centres researching lasers, space, manufacturing, groundwater, information technology, advanced materials and telecommunications. After the selections were announced, the panel's ratings of the proposals were made public, part of an attempt to show that political considerations did not influence the decision. The centres programme also reserves 22 per

cent of the allocated funds for indirect costs of university participation, a decision that greatly pleased university administrators.

Although the work of the centres will involve basic science, Peterson says the ultimate thrust of the plan is to create jobs in Ontario: Ontario's plans should really be national in scope, and if the centres are successful — and Peterson says the jury is still out on that — they may be extended outside provincial boundaries. □

\* *Competing in the New Global Economy. Report of the Premier's Council. Volume 1. Toronto, 1988.*

## Institutes at home in Toronto

### Toronto

NOT everyone likes to admit it, but no one denies that the University of Toronto (UT) is Canada's number one university. It is the largest university in Canada, receives the largest share of federal research money, and has a share in five of the seven Ontario Centres of Excellence. Pressed financially like every other Canadian university, UT is nevertheless in a good position to weather fiscal storms.

King's College, as UT was first known, received a royal charter in 1827, and courses were first offered in 1843. But in 1849, the provincial government took over the university, declaring the former Church of England-run institution non-



At the SHARP end — one-eighth scale model of the stationary high altitude relay platform.

denominational, and changing the name to the University of Toronto. Today the university has some 50,000 students, and 12,000 faculty members.

Last year was an especially good one for UT scientists. In February, UT astronomer Ian Shelton, working at the university's observatory in Chile, first identified what later turned out to be supernova 1987A, the most exciting astronomical event of the year. Also last year, UT chemist John Polanyi shared the Nobel prize for his work on chemical lasers. Polanyi is a member of the Ontario Laser and Lightwave Research Centre, one of Ontario's centres of excellence, headed by Boris Stoicheff.

UT has a number of special institutes.

The Institute for Aerospace Studies, for example, has its own building near the northern boundary of the city. The institute is home to an unusual assortment of research activities. James DeLaurier's work has led to the development of a unique, lightweight aeroplane, capable of being kept aloft by microwaves beamed to the plane from the ground. The one-eighth scale model is a prototype for a system called SHARP (stationary high altitude relay platform), a joint project with Communications Canada. While projects like DeLaurier's and others like Jørn Hansen's research on new composite materials for spacecraft and aircraft seem appropriate for an aerospace institute, others like J. Barry French's development of trace gas analysis devices or Peter San-geby's works on Canada's effort in fusion energy appear somewhat out of place.

A relatively new addition to UT is the Canadian Institute for Theoretical Astrophysics (CITA). Located on the downtown Toronto campus, CITA officially opened in 1984. Although only a small group, consisting of four faculty, CITA has achieved international recognition for its work. Peter Martin explains that the institute has helped keep theoretical astrophysics going in Canada, not just at UT but at smaller universities, through visiting faculty programmes. Martin also points out that theoretical work is cheap, compared to the more expensive hardware required by observational astronomers. But he is quick to support his observational colleagues' cause. Canadian astronomy has suffered some severe blows recently, from the closing of the Algonquin Radio Observatory to the decision not to fund the very long baseline array, eight radio telescopes that would have stretched from Newfoundland to Vancouver Island and given a resolution of 0.0004 arc seconds at 1cm wavelengths.

Biomedical Research at UT is spread among the medical school, the main campus, and eleven affiliated hospitals. Last year the university received some \$71 million in outside research funding, mostly from the Medical Research Coun-



## School and work

### Waterloo

THE University of Waterloo came into being 30 years ago, intending to accomplish two things; provide Canada with engineering and mathematics graduates, and offer a new approach to undergraduate education. In both it has succeeded, along the way gaining an international reputation for computer software products.

When Waterloo opened its doors in 1957, it was the first university in Canada to offer a cooperative education programme. Under this scheme, students split their undergraduate years between academic work and full-time employment in a field related to their area of study. At the end of the five-year undergraduate degree programme, by working summers a student will have completed the same number of courses as someone in a traditional four-year programme, but will also have two years of related work experience.

There are 15,000 full-time students at Waterloo, 9,000 of whom are in the cooperative education programme. For some areas of study, cooperative education is a requirement; for others it is optional. While employed, the student is paid a salary, and unacceptable job performance has the same effect as failing a course.

Waterloo's strength in computer science comes from a tradition of making computer literacy a priority. The university now produces about one quarter of Canada's PhDs in computer science. With Oxford University Press, Reed International and IBM, Waterloo is participating in a project to put the Oxford English Dictionary in machine-readable form. □

cil (MRC). The medical school has several strong departments, including medical genetics where Ron Wortman's group recently published details of the action of the gene responsible for Duchenne muscular dystrophy (see *Nature* 333, 466; 1988), and Roy Gravel has identified the gene defect in Tay-Sachs disease (see *Nature* 333, 85; 1988).

UT's biggest problem may be its own success. Having succeeded in garnering the largest share of granting council money, it is immediately set upon by less successful universities who accuse UT of hogging scarce resources. But the flip side of that argument, elaborated eloquently by Louis Siminovitch, director of research at the newly formed Mount Sinai Hospital Research Institute, is that regional demands have diluted the pursuit of excellence at what is clearly Canada's strongest university. Siminovitch is hopeful that Bill C-22 will make it easier to attract new research dollars from the pharmaceutical industry, but underfunding problems will persist for everyone, including the large universities, until Canada decides its research priorities. □

## Finding a new path to the top

### Toronto

FOR a country as large as Canada, promoting effective research collaborations among far-flung researchers is difficult, if not impossible. But the Canadian Institute for Advanced Research (CIAR) is committed to the notion that such collaborations can occur, given the proper encouragement, and that the effort will be worthwhile. CIAR is not yet a permanent fixture on the Canadian science landscape, but in its six years it has begun to chart a new course for the support of Canadian science. The concept behind CIAR is novel; to create a national institute not tied to any particular location, but



J. Fraser Mustard — stirring the pot.

capable of finding the very best Canadian researchers and giving them the funds and logistical support to work together from their home institutions.

J. Fraser Mustard is the driving force behind CIAR. A man of enormous energy, Mustard has stumped around the country, stirring the pot of Canadian research, and watching for the best ideas and best talent to rise to the top. The aim is to support areas of "intellectual, economic or social importance that involve the study of complex problems".

CIAR has no facilities and supports only people. In 1987 the institute spent \$2 million on salaries for 80 Canadian fellows, scholars and associates at 16 Canadian universities as well as the National Research Council and Statistics Canada. CIAR also supports 21 foreign researchers. Operating funds are raised from corporate sponsors and a \$7 million matching grant from the federal government. Mustard argues that left to their own devices, scientists will naturally form collaborations with like-minded colleagues. He acknowledges that formalizing this 'networking' process is a gamble, but one which will have a huge intellectual payoff if it works.

Mustard's own well-informed enthusiasm for Canadian science goes a long way towards selling CIAR. A fifteen-minute chat easily stretches into a two-hour conversation ranging from factors affecting

platelet aggregation — Mustard's own area of research — to strategies for improving Canada's economic competitiveness by supporting science.

But unlike many new schemes for supporting science in Canada, CIAR's emphasis is not on directed research, but on research excellence. Some programmes — such as the one on artificial intelligence and robotics — may well lead to marketable products, but neither the programme in cosmology nor the one in evolutionary biology have obvious economic spinoffs.

William Unruh, a cosmologist from the University of British Columbia, says that CIAR's programme has allowed Canada to woo back Canadian scientists from abroad. Ian Affleck from Princeton University and Richard Bond from Stanford University have both returned to Canada as CIAR fellows. The CIAR programme also allows Canada's relatively small cosmology community the opportunity to spend time together and develop a sense of identity. Affleck is now with Unruh at British Columbia, and Bond is in Toronto at the Canadian Institute for Theoretical Astrophysics (see p. 728).

Other active programmes of CIAR are in population health and superconductivity. Mustard expects two new programmes, one on law and society and another on technology, change and society, will be starting soon. The largest CIAR programme, with 37 members, is in artificial intelligence and robotics, led by Zenon Pylyshyn of the University of Western Ontario. The programme focuses on machine vision, knowledge representation and reasoning, and more recently advanced robotics.

Mustard likes to describe the research enterprise as a pyramid, with basic science forming the broad base. While CIAR emphasis is on the base of the pyramid, it is also involved in the next level up, where industrial applications are more tangible. CIAR has helped form PRECARN Associates, a company aiming to "sponsor, manage and disseminate the results of long-term, 'pre-competitive' research projects" in artificial intelligence and robotics. Gordon MacNabb, former president of the Natural Sciences and Engineering Research Council, is the president of PRECARN. So far, PRECARN has 35 corporate members, and MacNabb says he is finding it much easier to generate a consensus on research directions now that he has left the government. MacNabb reckons that unless PRECARN can do for Canada what Alvey, Eureka and Esprit have done for the United Kingdom and Europe, Canada will lose out commercially in an area where it has the potential to be competitive. □

# Separate but equal rules the day in forward-looking Quebec

## Montreal

A WALK through Montreal reveals not a single street sign in English. Even an English-language bookshop close to McGill University, where courses are taught in English, bears the sign *Bibliothèque*. In a city where English is widely spoken, this is the most conspicuous legacy of the landslide victory of the Parti Québécois, led by René Lévesque, in 1976. Although its commitment to an independent Quebec was abandoned after the 1980 referendum, the party's nine years in power helped bring about major changes in the status of the French language.

The changes came through Bill 101, the Charter of the French Language, designed to make French the language of legislation, law, administration, business and education. The bill was not well received by business, helping persuade several companies to move head offices from Montreal to Toronto. But it led to rapid promotion for bilingual Canadians, most of whom spoke French as their first language. That appears to have aided the rise of a dynamic French-speaking entrepreneurial culture. Gilles Cloutier, rector of the Université de Montréal, points out that Quebec now produces more than half of Canada's Masters of Business Administration. The income gap between francophones and anglophones has narrowed greatly.

Bill 101 introduced controversial changes in education. Only children who had received elementary instruction in English, in Quebec, could go on to English secondary schools; incoming children taught in English in other provinces had to switch to French. The aim was to ensure that the French character of Quebec could not be swamped by immigration. The section was ruled unconstitutional in 1984, but by then some of the steam had gone out of the great language debate, and the following year Parti Québécois was defeated by the Liberals.

In the meantime, the need for bilingualism, at least in eastern Canada, and the special nature of Quebec, had been much more widely accepted. Government officials in Ottawa had learnt to speak French, and services in French were made available wherever there was demand.

Many francophones seem to have taken a pragmatic attitude to the language. After Bill 101 there was a rise in the numbers of francophones at anglophone McGill University; up from 5 per cent in 1975 to 26 per cent now, probably because ambitious francophone students realized they would have to learn to speak English fluently sometime and university was a good place to begin.

With Quebec's separate culture better recognized, quarrels over language are more personal clashes than political clashes. A group of post doctoral students at McGill points out, with some anger, that even though their laboratory contains more francophones than anglophones, there is no possibility that French will be used in the laboratory. A researcher from the University of Laval recounts, with some anger, that when he worked in a laboratory in west Canada he was referred to as a "foreigner" — in his own country. A grants council panelist reports, with some anger, that a fellow panelist will not

give equal weight to recommendations written in French.

Incidents such as these will vanish as bilingualism triumphs and the choice between French and English loses emotional charge. The alternative, that French culture will slowly vanish from Canada, now seems exceedingly unlikely.

Two national characteristics may prove an unappreciated asset for a nation. Cloutier is an optimist, believing that Quebec is developing rapidly in the sciences and in business. He points out that the Université de Montréal has particular strengths in the humanities and social sciences, both areas in which French thinkers have had profound influence. "The French cultural background in the North American context could put Quebec in a position to lead", he says. □

## A contrast in styles

### Montreal

THE Université de Montréal is a complex of pale yellow art deco towers and steeples standing on top of Mount Royal, the hill that rises above Montreal's riverside business centre. Half a mile away, down the side of the hill, is a cluster of concrete buildings. These are the new laboratories of McGill University, founded in 1821, and now one of Canada's best known and wealthiest universities. Further down the slope are McGill's older buildings, including the physics building where, in 1901–2, Ernest Rutherford and Frederic Soddy performed experiments that led to the atomic theory and a Nobel prize.

McGill, an anglophone university in a sea of French-speakers, is thriving. New research centres, groups and units have been "springing up like mushrooms as people get fed up with the traditional divisions of departments", says Gordon MacLachlan, dean of graduate studies and research. There are now 74 in total; the number appears to be doubling every twelve years. Many bring together scientists from several different institutes; the

centre for plant molecular biology, for example, combines biologists from McGill, and biochemists from the Université de Montreal and the NRC Biotechnology Research Institute.

Over at the francophone Université de Montréal, the rector, Gilles Cloutier, wants no more formal research institutes for a while, "to avoid the balkanization of the university". The Université de Montréal has come through some difficult times, hit by the economic depression of the early 1980s and the provincial government's past emphasis on support for the newer universities. Cloutier became rector in 1985, facing a \$12 million deficit and a morose faculty. But he says now he "looks forward to the next few years". He has persuaded the provincial government to turn around its funding policy and he is successfully imitating McGill in a quest for large-scale private sector support. The budget is now balanced, a new younger leadership is emerging in the university and they are ready to expand by hiring "young research scientists of great calibre". □



Université de Montréal (left) and McGill's new laboratories.



# Healthy Québec Recherche

## Montreal and Quebec City

QUEBEC'S biomedical research enterprise is in good health. Part of the credit must go to the Fonds de la Recherche en Santé du Québec (FRSQ), a regional council supporting research in the biomedical sciences. FRSQ has been adding \$30 million a year to the Medical Research Council's \$50 million a year in federal funds spent on biomedical research in Quebec. The fund is envied; most provinces have nothing to match it. Quebec was the first province to have its own science policy, driven by a need to develop the francophone universities. But plans to complement FRSQ with grants councils for the other sciences have not materialized.

FRSQ does not replace MRC funding. It concentrates on training and start-up grants for young researchers (a condition of the latter being that operating grants have already been obtained elsewhere). FRSQ also encourages the establishment of research centres and institutes with interdisciplinary themes.

Montreal has Canada's largest concentration of bio-medical research institutes. In part this is due to the parallel development of English and French institutions; the hospitals of Hôtel-Dieu and Notre-Dame are matched by Montreal General Hospital. Each institution has spawned research centres.

Outside of Montreal, FRSQ has helped the growth of new centres. The Centre de Recherche du CHUL attached to the Université de Laval at Quebec City is a \$1 million a year beneficiary. There, Fernand Labrie has built a world-wide reputation for his studies in molecular endocrinology.

Close by, at Quebec City, the Hôtel Dieu, the oldest hospital in North America, has built up what must be one of the world's most crowded research institutes in the Centre de Recherche en Cancérologie de l'Université Laval. Its 100 staff, squeezing past equipment in narrow corridors and up and down steep staircases, tread the borderline between high mental stimulation and physical explosion. Astonishingly, ten years ago, when the director Luc Bélanger came here and began looking around for talented colleagues to join him, no research of note was being done.

That rapid growth is not uncommon, and there are many other groups in Quebec that have sprung up from the enthusiasm of individuals. Vitality, of course, creates a problem too: the number of groups trying to be serious players in the research scene has hugely increased — outstripping the ability of provincial and federal funding agencies to keep pace. □



Energy is one of Quebec's biggest exports to the United States, much of it generated at the stupendous hydroelectric complex at La Grande River, James Bay. Shown above is the spillway at La Grande 2. At time of flood, it draws off excess water from the dam and spends its force in a gigantic stairway of 13 steps, each 135 m wide and 10 m high. It can accommodate twice the flow of the St Lawrence River at Montreal. La Grande 2 has a capacity of 5,300 megawatts, a quarter of Quebec's total generating capacity.

## NRC's shot in the arm for biotech

### Montreal

A NEW addition to the Montreal science scene is NRC's Biotechnology Research Institute. It has a fine new blue building, complete with triumphal entrance arch.

The institute was set up by rejuvenating NRC biotechnology research teams with a large injection of fresh international talent. Average age of the new institute's 170 research staff is just 33 years. Industrial interest is high, according to director Bernard Coupal, with more demand for collaborative projects than the institute can easily handle.

Space within the institute has been rented out to five companies to help forge close links. The main research areas are in biochemical engineering, genetic engineering, protein engineering, and molecular immunology. Several projects have particular relevance to Canada's agriculture: the bioconversion of lignin, a by-product of the pulp and paper industry, and the isolation and engineering of insecticidal toxins from bacteria.

One problem for the institute is that the young superstars of biotechnology are in great demand, and salaries have come to really matter. The institute finds it hard to bid against flexible US companies and universities when its own decision-making powers are limited by red tape from Ottawa. The institute can give researchers security but as Coupal says, "If you're good you don't need security". □

## Tall family trees grow in Quebec

### Montreal

A FEW years ago every Canadian with the surname *Roberge* was invited to a party on the Isle d'Orléans, below Quebec City in the St Lawrence River. Four thousand two hundred people turned up, all descendants of two brothers who had started farming on the island in 1669.

One still holds the original land. The majority of French Canadians are descendants of just 8,000 settlers who came to Canada in the seventeenth and eighteenth centuries.

That, coupled with intact church baptism records going back to the seventeenth century, makes Quebec a good place for tracing ancestors. A computerized data bank of 660,000 baptisms, spanning eight generations, from 1842 to 1971, has already been established for the Saguenay region by a group of human geneticists. Their objective is to explain why particular genetic disorders occur often in some parts of Quebec.

Earlier, in 1969, the universities of Montreal, McGill, Laval and Sherbrooke had reacted to the high frequency of hereditary diseases with a demonstration

genetic screening project. That grew into the Genetic Medicine Network of Quebec: the world's first integrated screening, diagnosis, treatment and research programmes for genetic disorders.

Charles Scriver of McGill University was one of the key figures behind the scheme. He sees the present network as a model for a system that could cope with a larger spectrum of genetic influences on disease. Molecular diagnostics, he believes, will transform medicine as powerfully as did the X-ray machine. But he finds it "painful" that the government's research priorities are increasingly short term; funds for preventive medicine are harder to come by than twenty years ago.

Similar screening programmes have been set up in other provinces. Coupled with a universal health insurance system, which makes it easy to find relatives under the care of other doctors, Canada is fast becoming the world's major source of human genetic data. Of *Nature's* ten major publications in the field in the past two years, nine — spanning Tay-Sachs disease to retinoblastoma — use data from Canada. □

# Scientific research north of the sixtieth parallel

## Tuktoyaktuk and Ottawa

IN early May, the pace of life at the Polar Continental Shelf Project (PCSP)'s Tuktoyaktuk base is pretty slow. The Mackenzie River is still frozen, but the ice highway to Inuvik 130 miles to the south is closed because of an early thaw. The dormitory is empty, except for one or two pilots detailed to the base. Outside base manager Eddy Chapman's window, a helicopter sits to the side of the gravel runway of Tuk airport, and the radios at the head of Chapman's bed are silent.

But by mid-June, the situation will be quite different. Chapman will share with PCSP's base at Resolute the responsibility for nearly 1,100 researchers in 50-60 camps, spread over an area the size of the entire eastern United States. Working in the Arctic has lost some of its frontier qualities — with snowmobiles and television via satellite now a routine part of life — but veterans like Chapman know that care is still needed to negotiate the far north safely.

A concern to show to the world that Canadians work in the Arctic and that the Arctic belongs to Canada has been a major force in pushing up budgets for Arctic research. That, in turn, has made the Arctic a great deal more accessible; a scientist in a laboratory in Montreal or Toronto can, a day later, be at home on an ice island, floating in the Arctic Ocean, without having suffered any more discomfort than a wait to change planes at Resolute base. Still the excitement of the Arctic remains.

"To me the Arctic is a paradise", says Pierre Lapointe, PCSP director. PCSP

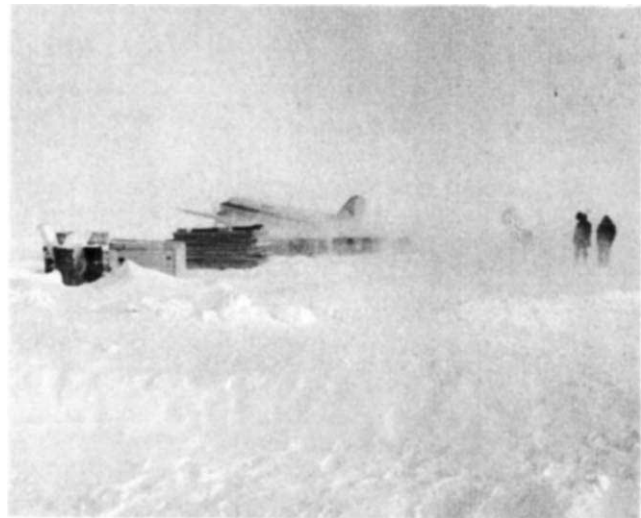
has made routine Arctic travel possible. Despite his administrative duties, Lapointe spends a couple of months a year in the Arctic, although he complains it is not nearly enough.

PCSP celebrates its thirtieth anniversary this year. An independent body within the Ministry of Energy, Mines and Resources, PCSP provides logistic support and coordination for arctic research free of charge to the universities and to government research departments and on

precisely-surveyed baselines from which Decca radio-navigation equipment could be run. Now, satellites make it much easier and a simple transponder can fix locations exactly. PCSP's fleet of 5 Twin Otters, 20 helicopters, a DC3, a 748 and a Hercules (all rented) has to be carefully distributed. Fuel is cached all over the Arctic.

Lapointe is proud that a scientist has never been lost. Every morning and evening each camp is contacted by radio either from Tuk or Resolute base. If three calls in a row remain unanswered then a rescue plane is despatched. Chapman says researchers have been known to lose track of time, and forget to call in. But since the researcher must reimburse PCSP at \$840 an hour for an unnecessary Twin Otter trip, the mistake is rarely repeated.

The Arctic is a busy place in summer. For the 1988 field season 227 projects have been planned. Among them biologists will be looking at Beluga whales at Ramsay Island, narwhales in Eclipse Sound, bearded seals at Eskimo Point, and listening to the sounds of undersea mammals at Admiralty Inlet. Geologists will be visiting fossil forests on Axel Heiberg Island and collecting meteorites on



*A Douglas DC3 lands on the ice island.*

a cost-recovery basis to other clients. Its annual budget was boosted from \$5.2 million to \$7.4 million for this fiscal year.

The key to managing so many scientists in the field is knowing exactly where they are. In the earlier, heroic days of the project, the top priority was establishing

Ellesmere Island. A handful of archaeologists will be searching for the remains of old Inuit settlements.

The Ministry of Indian and Northern Affairs also offers research services to scientists. With base stations in Inuvik in the west and Igloolik in the east, the Northern Scientific Resource Centres, directed by David Sherstone, will offer support for another 250 scientists.

Scientific work in the Arctic has shifted from the dizzy excitement of exploration to more sober scientific research. The scientific questions being asked about population size in the Arctic, for example, are the same questions being asked about populations further south. Greater demands on PCSP's resources means only good science can be supported.

PCSP's most peculiar base is on an ice island floating in the Arctic Ocean. Right now, the island is somewhere around 80°N, 106°W; that is, off the coast of the Svedrup Islands. Sitting on it are a group of 17 huts containing living quarters and laboratories for 30 scientists and an airstrip which allows planes to land after a three-hour flight from Resolute.

No one is certain where the ice island will go next. Sometimes it moves at 850 metres per hour for a week and sometimes it stands still for a week. But the betting is



*A life on the ocean waves. Seventeen huts are clustered on an ice island off the Svedrup Islands. The ice island is 5 years old and may last another 40 years.*





Offshore Arctic drilling. Conditions are the most rigorous yet tackled, but rewards could be worth it.

that it will go somewhere interesting, probably down the west coast of the Arctic islands to the Beaufort Sea, then along the Arctic shelf and back up into the Arctic Ocean to circulate around the North Pole. All that time — 20 or even 40 years — scientists hope to be transported free of charge over some of the least studied ocean in the world. On board are ocean bottom samplers, and also equipment for continuous seismic reflectance studies.

The island is nearly five years old. It calved from the glaciers on north Ellesmere Island in late 1985 and, being made of freshwater ice, rather than sea ice, is strong enough to survive in the ice pack. It is also very big: two and a half kilometres long, a kilometre wide, and a thousand million tonnes in weight.

There is a chance the island may wander into the Soviet side of the Arctic. That is not a problem, jokes Lapointe, because the island can always be registered as a ship and it will be in international waters. He hopes that the Americans and Soviets will help to provide access to the island and its availability will stimulate international cooperation in Arctic research. The early signs are apparently very encouraging.

A better understanding of ice, and what can and can not be done with it, has enabled a remarkable oil exploration project to proceed in the Beaufort Sea north of Tuktoyaktuk. BeauDril Ltd, a partnership of several companies led primarily by Gulf Canada Resources, has shown that it is possible to drill for oil year round in the

Beaufort Sea icepack. Using the *Molikpaq*, a mobile caisson built in Japan for use in the Arctic, to drill test wells, BeauDril believes it has found a commercially viable oilfield at Amauligak, 65 km off shore.

The *Molikpaq* was towed to its current location, where it was lowered onto a base of sand on the ocean floor. The core of the *Molikpaq*, the area of two football fields, was filled with more sand, to a depth of some 22 metres. As ice flows towards the caisson, it grounds out on the sand platform that the *Molikpaq* sits on, creating a rubble field that prevents other ice from hitting the caisson.

BeauDril learned some lessons about ice the hard way. A smaller drilling platform, called the *Kulluk*, uses ice breakers to reduce approaching ice to a manageable size. But BeauDril overestimated how much ice the *Kulluk* could stand, and it was necessary to terminate drilling operations abruptly before *Kulluk* was parted from the twelve anchors that keep it in place. The only real option when big ice is coming is to get out of the way.

BeauDril uses synthetic aperture radar developed by MacDonald Dettwiler (see p.726) to track ice movement under the arctic cloud cover.

BeauDril is still trying to determine whether the Amauligak oilfield is large enough to justify commercial operation. If it is, then a new national debate will have to start about whether to build a pipeline to carry the oil through the Northwest Territories to join up with the existing pipeline in Alberta. □

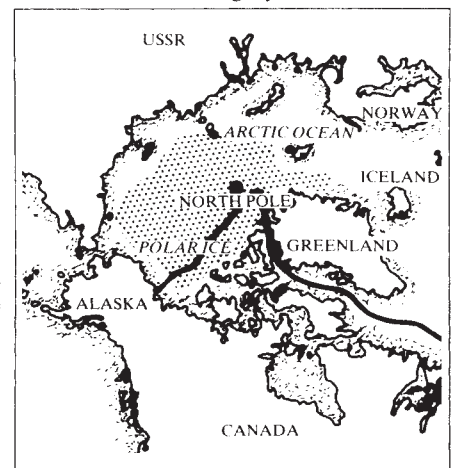
## Sovereignty is still a source of tension

THE map below shows the enormous area of the Arctic occupied by Canada's 200 mile economic zone; if sovereignty were extended to the edge of the continental shelf the area is even bigger, going all the way up to the North Pole. The Arctic is rich in mineral and oil deposits.

But Canada has nothing to defend this territory, "not so much as a canoe" as Prime Minister Mulroney once put it. So Canada is now considering spending \$7,000 million on a fleet of nuclear-powered attack submarines, capable of operating under the Arctic ice throughout the year. A giant "Class 8" ice breaker is also to be ordered from a British Columbia shipyard.

The official threat to Canadian sovereignty is the Soviet submarine fleet. But it is incursions by US vessels that have whipped the Canadian government into action. US submarines have passed between Canada's Arctic islands and, in incidents that inflamed the Canadian public, an ice-strengthened US oil tanker, the *Manhattan*, passed through the Arctic islands in 1970, followed by the US Coast Guard ice breaker *North Star* in 1985. In neither case did the US government ask the Canadian government's permission for the voyages.

The United States and Canada signed an Arctic Cooperation agreement in January this year that should prevent further incidents. But the US side would not concede sovereignty over the famed



north-west passage which winds among Canada's islands from the Prince of Wales Strait to Lancaster Sound, even though the passage is only twenty miles wide at its narrowest. The United States regards the waterway as international, saying that if it yielded to Canada it would have to yield navigation rights in narrow straits elsewhere in the world. □

## Alberta hopes to keep the wheels of research well oiled

### Edmonton

OIL has played a vital role in Alberta's fortunes. A decade ago when oil prices were high, the government amassed some \$13,000 million in surpluses. But when the price of oil declined, the pace of development slowed, and the government began to seek a return from those on whom it had lavished support. Although Alberta is seen by the rest of Canada as the land of plenty, the belt tightening of the past few years has brought on the same problems felt around the country.

Alberta has concluded that one key to prosperity is to attract new, high-technology industry. To accomplish that, the government established a ministry of technology, research and telecommunications. Officials at the ministry feel they have a right to ask universities to be more responsive to applied research opportunities. They like to point out that the University of Alberta and the University of Calgary are two of three universities in Canada that get more than 40 per cent of their research budgets from provincial governments.

Although the universities agree in principle with this desire to transfer technology from universities to industry, University of Alberta president Meyer Horowitz accuses the government of pointing only to research dollars, which have risen, while ignoring cuts in other areas of university support. These differ-

ing points of view have raised tensions between educators and government officials.

Alberta has a long history of support for science. The country's first agriculture station was established in Lethbridge around the turn of the century, and in 1921 the provincial government established the Alberta Research Council as a crown corporation.

Today the council has a budget of approximately \$45 million, and projects supported range from biotechnology to coal and hydrocarbon processing, always with an eye to commercial use.

The Alberta Heritage Foundation for Medical Research was set up in 1979 with a \$300 million endowment, a move to create a cadre of top medical researchers in the province (see below).

Alberta has also sought to make a mark in the advanced electronics world. The Alberta Telecommunications Research Centre and the Alberta Microelectronic Centre hope to attract new industry by forging cooperative links with Alberta's universities. LSI Logic of Milpitas will soon be taking over a large research facility recently abandoned by Bell Northern Research where it will pursue work on application-specific integrated circuits. There is also a laser institute, a supercomputer centre boasting a CDC Cyber 205 supercomputer, and a biotechnology pilot plant. □

## Looking to the east for herbal remedies

### Edmonton

ALTHOUGH herbal compounds have been a part of Chinese medical practice for 2,000 years, it is only in the past few decades that China has made a serious attempt to purify them and classify their active ingredients. At the University of Alberta, Peter Pang has established a collaboration that has brought the eastern pharmacopoeia under the scrutiny of western pharmacological techniques.

Pang set up the first collaborative programmes with Chinese researchers in June of 1986. He has now established eight different collaborations, and travels to China several times each year to monitor their progress.

Of particular interest to Pang are herbal calcium antagonists used as antihypertensives. Although his work is not strictly aimed at drug development, he has received a \$1 million grant from an international pharmaceutical company, that has been matched by a \$700,000 grant from the Natural Sciences and Engineering Research Council.

Pang's group will be primarily concerned with determining the mechanism of action of herbal compounds. Pang says this is the flip-side of rational drug design. Rather than creating a promising compound and then testing it in animals and humans, Pang is looking at compounds that have already had 2,000 years of testing for toxicity and efficacy. Now, he says, they just have to figure out why they work. □

## Firm medical foundation

### Edmonton

Nor yet ten years old, the Alberta Heritage Foundation for Medical Research (AHFMR) has already had a profound impact. AHFMR funds have begun to transform the University of Alberta medical school from a somewhat sleepy, clinically oriented programme into a major force in Canadian biomedical research. The University of Calgary, too, has benefited. That AHFMR has accomplished this is perhaps not surprising, given its deep pockets from a \$300 million endowment. More surprising is that the foundation exists at all.

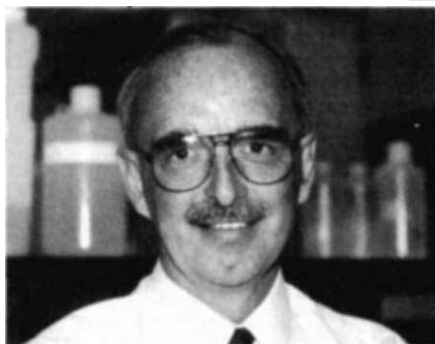
With oil revenues running high in the 1970s, Alberta had to find a politically acceptable way to dispose of its surpluses. Lionel McLeod, then dean of the University of Calgary medical school, led a group that urged the province to endow a medical foundation that would both improve the stature of research in the province, and improve the quality of health care. In 1979 the provincial government created AHFMR as an entirely independent organization, required only to report to

the legislature at annual intervals.

A large fraction (45 per cent in 1986) of AHFMR's spending goes on research support and institutional grants to the University of Alberta and the University of Calgary. To attract new faculty, the foundation guarantees five years of salary support, and substantial sums for equipment purchases. An additional ten years of foundation support is contingent on performance. Over 100 scientists have been recruited using these funds. The foundation also has a number of graduate and postgraduate student support programmes. Foundation awards are reviewed by a panel of international scientists.

C. R. (Bob) James, vice-president for research at the University of Alberta, says AHFMR's potential for promoting change is easy to see if it is compared to federal support of medical research. The foundation spends \$60 million per year on Alberta alone, whereas the MRC budget is \$180 million for the entire country.

There are still problems, however. Apart from the Edmonton Oilers hockey team, Edmonton offers little in cultural



Alberta Heritage aid continues to bring smiles to researchers' lips. "We can still dream dreams, and they can come true", says biochemistry department chairman William Bridger.

entertainment, and does not have the natural beauty of the Rocky Mountains as a backdrop as does Calgary to the south. Scientists are often enthusiastic about the university, but spouses veto the move.

There are also limits to AHFMR's largesse. Commitments to two new research buildings under construction in Edmonton and Calgary, coupled with declining interest rates and more conservative fiscal plans, are forcing the foundation to slow down spending. □



# Space just another frontier for imaging-conscious Canada

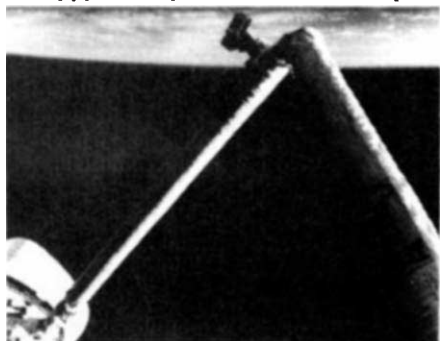
## Ottawa

ALTHOUGH it is a high stakes game, Canada has spent generously on space. The government will contribute \$1,200 million to the International Space Station, and has plans to launch sophisticated remote sensing and communications satellites within the next six years. But coordination of space activities is fragmented, budgets have not been stable, and international partners have failed to climb on-board some projects as expected.

One of Canada's most visible successes is the remote manipulator arm on the US Space Shuttle. It may have been US astronauts on a US spacecraft that captured an errant satellite but it was the Canadarm, with a maple leaf and the word "Canada" emblazoned on the side that took the satellite in its grasp, keeping it from floating off into space. Canadian space officials speculate that US Congressman were not pleased by this sight. Even though Canada had proven its expertise with the Canadarm, there was resistance to allowing Canada to contribute the mobile servicing system (MSS) for the space station.

Like other countries, Canada had its share of difficulties in negotiating an agreement with the US National Aeronautics and Space Administration (NASA) for participation in the space station.

Unlike other countries, who are building separate modules, the MSS is part of the station's infrastructure. If Canada is unhappy with a potential use for the space



The space shuttle's remote manipulator arm, viewed from Columbia's flight deck.

station — such as a Pentagon project — there is not much Canada can do about it. Other countries can essentially 'shut the door' on their modules if they are unhappy.

Earlier this year Canada's participation in the space station was jeopardized when the government admitted that it was \$400 million short of the funds necessary to complete its part of the construction. But with \$184 million taken from the \$1,300 million committed for science and technology (see page 723), and additional money promised but not yet identified, the project is moving forward.

MSAT, a satellite designed for two-way radio and telephone communications for users all over the country was scheduled for launch in 1990. The federal government will lease MSAT services from Telesat, Canada's commercial domestic communications satellite company. Getting the final go-ahead for MSAT has been contingent on frequency assignments with the US Federal Communications Commission, a headache that has yet to be cured.

The diversity of space projects points out a problem with Canada's space activities. Communications Canada is the lead agency for MSAT, Energy Mines and Resources is the lead for RADARSAT, and space station activities are led by the National Research Council.

Plans to form a space agency have been held up by a geographical dispute. Although the small group handling space activities for MOSST is now in Ottawa, Montreal has lobbied hard to have the new agency located there, coming up with the slogan *Montréal est spatiale* to push its claim.

Space science has not fared quite as well as space hardware development. NRC closed the rocket launch facility in Manitoba in 1984. Still, space institutes at the University of Toronto and York University in Toronto are doing well, and Canada will have an active scientific role on the space station when and if it is launched. Canada maintains an astronaut corps, and Canada is entitled to have one astronaut on board the space station for six months every two years.

Remote sensing has been a Canadian strength. With huge areas of forest and tundra that have never been visited, let alone accurately mapped, Canada was quick to exploit the opportunities offered when Landsat was launched in 1972. A Canadian-built receiving station was ready on day one, and the world's first centre to promote the use of satellite data was in business soon after. Now it transfers both French SPOT satellite data and Landsat data to a host of provincial and commercial users. But "optical inhospitality", near continuous cloud cover over much of Canada's least-charted northern regions, means that supplementing optical data with radar mapping made the most sense for the future.

Demand for radar surveying has spawned a couple of Canadian success stories. An Alberta-based company, Interra, made a reputation in radar mapping from aeroplanes, capturing over 90 per cent of the world market. MacDonald Dettwiler, from British Columbia, set new standards in real-time processing of syn-

# Canadians can phone home from anywhere

## Ottawa

RUNNING a country the size of Canada requires an efficient telecommunications system, and Canada has one of the best. Even tiny communities inside the Arctic Circle have instant communications with anywhere in the world. Communications Canada reckons that 98 per cent of Canadian homes have telephones, the highest percentage in the world.

There are two major government laboratories devoted to communications research activities; the David Florida Laboratories at Shirleys Bay near Ottawa, Ontario, and the Canadian Workplace Automation Research Centre in Laval, Quebec. The David Florida Laboratories also have extensive satellite testing facilities. Earlier this year the European Space Agency communications satellite Olympus was at Shirleys Bay for systems checks.

The research laboratories are currently developing a teletext system that may be used for all government telepicture and database systems. In addition to research in advanced semiconductor materials, the communications research laboratories have collaborated with the University of Toronto to produce a microwave-powered aircraft (see page 729) that would perform many of the same tasks as a communications satellite, but at a fraction of the cost. The one-eighth scale model that flew last year is quite literally 'mothballed' at the David Florida Laboratories. Last winter it was discovered that mice had eaten the balsawood frame of the aircraft during the winter. Mothballs are keeping potential diners away from the rebuilt model. □

thetic aperture radar images from the US SEASAT satellite. These successes helped create a consensus to build RADARSAT, a satellite carrying a synthetic aperture radar capable of mapping through cloud and in darkness.

Seven years into the project, the future of RADARSAT is unclear. RADARSAT's instruments were supposed to go on a platform provided by the British. But Britain decided to support a polar orbiter being planned by the European Space Agency instead, leaving Canada searching for a way to build the satellite using the \$400 million that has already been pledged by the federal and provincial governments and industry.

The aerospace industry is negotiating for the purchase of a smaller, less expensive platform than the one the British were planning to supply. MOSST has also made contact with other countries, notably Japan, to solicit interest in joining the RADARSAT project. NASA has promised to launch the satellite on a Titan rocket in 1994. □

# AECL seeking a renaissance for nuclear power exports

## Ottawa and Mississauga

FOR a country so rich in coal, oil and hydroelectric power, developing nuclear power may seem unnecessary. But Canada also has rich supplies of uranium, and since 1952 Atomic Energy of Canada, Ltd (AECL) has led a vigorous programme of reactor development, motivated in part by the desire to show Canada can master high technology. The chief result has been the series of Canada Deuterium Uranium (CANDU) reactors.

AECL is a crown corporation. That means that, in the end, the government is responsible for its debts. Without that kind of backing, potential foreign customers for CANDU reactors would probably not have taken the programme seriously. Nuclear power development is a long-term, highly competitive business. In the United States and Japan, nuclear power development could be left to giant, secure engineering companies. In Canada the government had to be involved.

In 1985, the government began to reduce its obligations. CANDU had not been a failure; five power reactors had been built in Canada — the three largest in Ontario — and six overseas. But it had not succeeded in winning a single sale in the United States, France, Britain or Japan and there seemed no market for CANDU left.

AECL's research operation, most of it concentrated at the massive Chalk River Nuclear Laboratories was scheduled to be cut in half over three years. Some of the loss was made up by money from Ontario Hydro which was the main utility to have profited from the nuclear programme. The CANDU commercial operations programme was cut back drastically, and plans made to privatize AECL's radiochemical operation.

AECL intends to diversify its operations and get into industrial areas like food

irradiation and accelerator design. It has also been dreaming up a smaller nuclear power plant that could be built more efficiently and regenerate international enthusiasm.

To accomplish these changes, AECL turned to computer-aided design systems (CADS) to begin building the CANDU 300. Gerrit Van Geyn, director of computer aided engineering for CANDU operations says it wasn't until 1987 that using CADS became cost effective.

There are about 45 different programme units needed to model the entire reactor, and about one third of these are now working well enough to replace traditional drafting techniques.

The idea is to use computers not only to come out with an effective design, but one that can be translated easily and efficiently to a construction schedule. Using CADS will also cut down on white-collar labour costs, since engineering drawings can be generated by the computer.

When built, the CANDU 300 will provide between 420 and 470 megawatts of electricity — the difference determined by the temperature of the cooling water at a potential location. AECL officials hope a reactor of this size will fit more easily into existing power grids than the larger CANDU 600.

Until CANDU 300 comes out of the computer's memory, AECL will be kept cheerful by sales of its "Slowpoke" system, practically the world's first home nuclear reactor. It consists of little more than a lump of uranium that heats water in a stainless steel tank. Enough heat is generated to provide for a large building or a small community. The system is completely safe and claimed to be cheaper than oil, gas or electric central heating. It requires no maintenance. Once every two or three years someone will come by to put in a new 800 kg lump of uranium. □

# Last acid words

## Ottawa

IT may seem strange that the very last article in this supplement on Science in Canada should concern acid rain when, in the minds of many Canadians, it is the single most important scientific issue of the day.

The tragedy is that there is little progress to announce: acid rain falls, maple trees die, salmon vanish from rivers, Canada protests to the United States about trans-border pollution, and the United States remains unwilling to pay the gigantic costs that immediate remedial action would require.

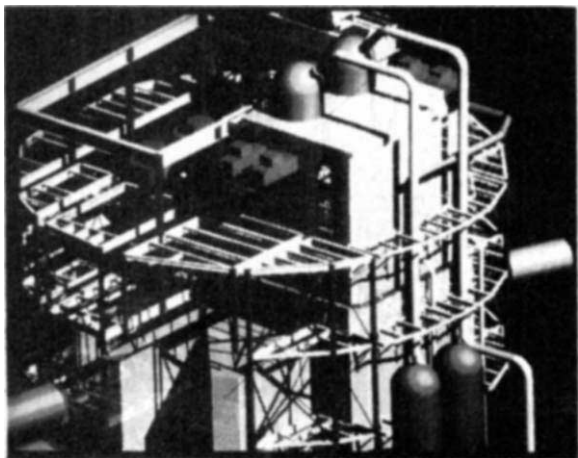
Impatient officials in Ottawa occasionally say its time to get tough with the United States. But when asked what that means, they have no reply. With the balance of trade at present running in Canada's favour, there is little that the United States could be denied to put on pressure.

Prime Minister Brian Mulroney knows this well. On his recent visit to Washington he made a great show of cheerfulness over President Ronald Reagan's promise that a proposed agreement to reduce acid rain would be "taken up as a matter of priority".

Mulroney believes that eventually the US administration will be persuaded of the justice of the Canadian case. Canada has already enacted legislation to halve emissions of sulphur dioxide from its own power stations by 1994.

Canada's acid rain woes do not originate exclusively with her neighbour to the south, however. In winter, a high pressure system in Siberia brings sulphur- and nitrogen oxide-laden air from Eurasia, so in February the pollution levels are typically higher at the Arctic station at Alert than they are along Lake Ontario. Another problem looming is 'toxic rain'. Scientists at Environment Canada are now finding polychlorinated biphenyls in northern snowpack.

With these problems before it and around one-fifth of the world's supply of fresh water to protect, Canada has begun to take a serious role as world atmospheric conscience. The treaty to limit the output of chlorofluorocarbons was signed in Montreal last year. Next week, Toronto plays host to the World Conference on the Changing Atmosphere, intended to usher in an International Law of the Atmosphere. Although the conference is sponsored by the Canadian government it is not intended to be a political event. Up for discussion are the depletion of the ozone layer, greenhouse warming and the way they might alter the Earth and planners' plans for it. High-level government officials will attend from many nations — but not from the United States. □



*Though missing out to Westinghouse on export orders to France and Great Britain, CANDU-type reactors are running in India, Pakistan, Argentina, Korea and Romania. CANDU 300, smaller than the established reactors, may improve export prospects.*



# Targeted misexpression of a *Drosophila* opsin gene leads to altered visual function

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*Drosophila* mutants transformed with a chimaeric gene that expresses the ocellar visual pigment in the major class of photoreceptor cells of the retina were used to investigate the properties of this minor pigment. The photoreceptor cells in which this opsin was misexpressed showed new spectral characteristics and physiology.

IN both vertebrates and invertebrates, photoreceptors express specific genes encoding proteins involved in phototransduction and spectral sensitivity<sup>1</sup>. One such set of genes codes for the cell-specific visual pigments. The genes encoding five vertebrate<sup>2-4</sup> and four *Drosophila* opsins<sup>5-10</sup> have recently been isolated and characterized. One of the *Drosophila* opsins, (Rh1), is expressed in the outer six photoreceptor cells (R1-6) of each ommatidium<sup>5,6</sup>, two others (Rh3 and Rh4) are expressed in non-overlapping sets of central R7 photoreceptor cells<sup>8-10</sup>. The remaining opsin (Rh2) is not expressed in either the R1-6 or R7 photoreceptors, but may be expressed in some R8 cells<sup>7</sup>. Through the use of *in vitro* mutagenesis and P-element-mediated DNA transformation, visual pigments normally expressed only in a small fraction of the fly's photoreceptors can now be uniquely expressed at high levels in the R1-6 cells which dominate the behavioural, spectral and photochemical properties of the eye<sup>11-13</sup>. Thus, by using such genetically-engineered *Drosophila* strains with cell-specific misexpression of particular opsin genes<sup>14</sup>, we are now in a position to study the photochemical and physiological properties of these minor visual pigments, and the behavioural consequences of their expression in different photoreceptor cells.

Most of the *Drosophila* opsins except for Rh1, which was first identified by mutations in the *ninaE* locus<sup>15</sup>, have been identified solely on molecular genetic criteria<sup>5-10</sup>. The Rh2 visual pigment is a good example of such molecule: it was identified and isolated by cross homology to an Rh1 complementary DNA probe<sup>7</sup>. In the present study, we have analysed flies in which the promoter of the Rh1 opsin was linked to the structural gene of Rh2 (ref. 14). This fusion construct has been reintroduced into the germ line of mutant host flies (*ninaE*<sup>117</sup>) carrying an internal deletion of the endogenous *Rh1* structural gene<sup>6</sup>. The only visual pigment expressed in the outer six photoreceptors of the transformed flies is the one driven by the newly introduced chimaeric gene. By expressing and studying the Rh2 photopigment in R1-6 cells we are thus able to determine whether its spectral characteristics match what was known about the R8 cells, or whether it represents a new opsin.

This general strategy of misexpressing components involved in phototransduction allows one to dissect which properties of a photoreceptor are intrinsic to it and which are a function of the genetically transplanted component. For example, R1-6 and R7 cells all show prolonged depolarizing afterpotentials (PDAs) when there is a significant net conversion of rhodopsin to metarhodopsin<sup>16-18</sup>. This phenomenon, however, has not been seen either in R8 (ref. 17) or ocellar photoreceptors<sup>19</sup>. Is this a property of the visual pigment or the downstream transduction machinery? The UV-sensitizing<sup>20</sup> and the long-wavelength absorbing pigments<sup>21</sup>, which are present in only certain sub-

classes of photoreceptors, lead to changes in the spectral sensitivity of the cell in which they are expressed<sup>22-24</sup>. Can these molecules work with any visual pigment or are they specialized to interact with only one?

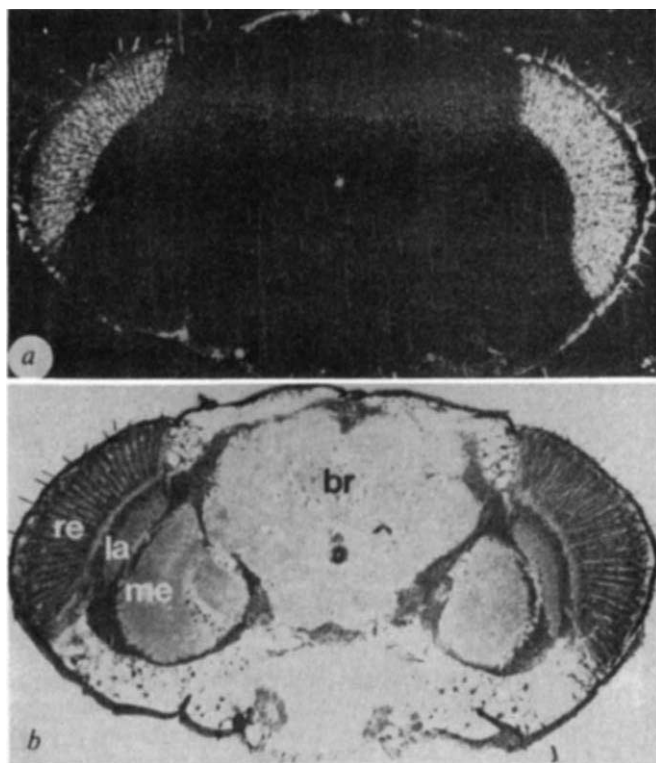
In this article we use high resolution microspectrophotometry and physiological recordings to determine the spectral sensitivity and photochemistry of the Rh2 visual pigment misexpressed in the R1-6 photoreceptor cells. Also, we have studied the visually guided optomotor behaviour of the transformed flies. Finally, we have used spectral measurements and RNA expression data in various mutant strains to demonstrate that Rh2 corresponds to the ocellar photopigment. In related studies, Pollock and Benzer<sup>25</sup> and Misner *et al.*<sup>26</sup> have recently shown that the *Rh2* gene is indeed expressed in the ocelli.

## Cell-directed misexpression

*Drosophila* transformed with chimaeric genes consisting of transcriptional fusions between the promoter element of the *Rh1* gene and unrelated reporter sequences express the reporters only in the six outer photoreceptor cells of the adult retina<sup>27</sup>. Zuker *et al.*<sup>14</sup> have recently generated flies transformed with a P-element vector containing a fusion between the Rh1 promoter and the structural gene encoding the Rh2 visual pigment. These flies, *ninaE*;P[*Rh1+2*] have a deletion of their endogenous *Rh1* gene (*ninaE*<sup>117</sup>)<sup>6</sup> and now functionally express the *Rh2* gene instead of *Rh1* in the R1-6 cells. Figure 1 shows an *in situ* hybridization to a frozen tissue section through the head of a *ninaE*;P[*Rh1+2*] fly, demonstrating that Rh2 is a major transcript in their R1-6 photoreceptor cells.

## Photochemical properties

*Drosophila* visual pigments, like those of most invertebrates, are photoconvertible from a photoactive rhodopsin form (R) (also referred to as xanthopsin<sup>28</sup>) into a thermally stable metarhodopsin from (M) (or metaxanthopsin). For the major visual pigment, Rh1, the R-form absorbs maximally at 480 nm and interconverts with an M-form which absorbs maximally at 580 nm (ref. 22). To determine the spectral absorption maxima ( $\lambda_{\max}$ s) of these two states for the Rh2 photopigment, we carried out microspectrophotometric measurements through the eye of *ninaE*;P[*Rh1+2*] flies. All experiments were carried out on white-eyed flies (genetic background *w*<sup>1118</sup>) to eliminate any effects of screening pigments. We relied on the generation of difference spectra which compares absorption properties after differential bleaching near the two (R and M)  $\lambda_{\max}$ s of the visual pigment molecule. We found (by trial and error) that maximal difference spectra (Fig. 2) were produced when 524 and 402 nm were the two bleaching wavelengths. With the same protocol,

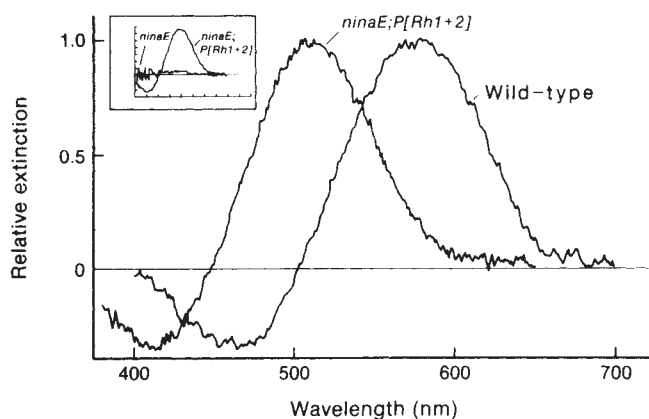


**Fig. 1** *In situ* localization of Rh2 transcript in *ninaE*;P[*Rh1*+2] flies. A 600-nucleotide *Eco*R1–*Bam*H1 restriction fragment of Rh2 was radiolabelled and hybridized to frozen tissue sections of adult *ninaE*;P[*Rh1*+2] heads as described previously<sup>7</sup>. Panel *a* shows a darkfield view of a cross-section with a strong signal over the retinas of the compound eyes, demonstrating high levels of Rh2 expression in the six outer photoreceptors (R1–6) of each facet. Panel *b* is a brightfield view of the same section indicating the location of the retinas, optic lobes and brain. Note that the signal in *a* is present only over the retina. Abbreviations: la, lamina; me, medulla; br, brain; re, retina.

untransformed *ninaE*<sup>117</sup> flies showed no difference spectrum (Fig. 2, inset). This is consistent with the fact that they contain no visual pigment molecules in their R1–6 cells<sup>6</sup>. Normal flies or flies transformed with a wild-type copy of the *Rh1* gene (data not shown), display a difference spectrum shifted considerably to the longer wavelengths compared to that of Rh2. The relative extinction spectra of the two Rh2 pigment states (R and M) (Fig. 3) were determined from the difference spectra as described in the figure legend. These curves show that the photoactive R-form (confirmed by sensitivity measurements below) of the pigment has a peak sensitivity at ~420 nm whereas the M form has a maximal absorption near 520 nm. Thus it is clear that the Rh2 photopigment has distinct photochemical properties from Rh1, and that it is therefore a novel but functional photopigment.

### Photoreceptors with misexpressed opsin

Having determined the spectral properties of the Rh2 photopigment *in situ*, we carried out electrophysiological recordings of the light-evoked response in *ninaE*;P[*Rh1*+2] transformed flies to determine spectral sensitivity. Figure 4 shows spectral sensitivity of control flies and of transformed flies using the 'light clamping' technique of Franceschini<sup>29</sup>. Consistent with the microspectrophotometric analysis, flies expressing Rh2 opsin in their R1–6 cells show a broad peak of sensitivity near 420 nm. In contrast, control flies show a sensitivity peak near 480 nm. This result confirms that the 420-nm absorbing form of Rh2 corresponds to the visually active (R) state of the pigment.

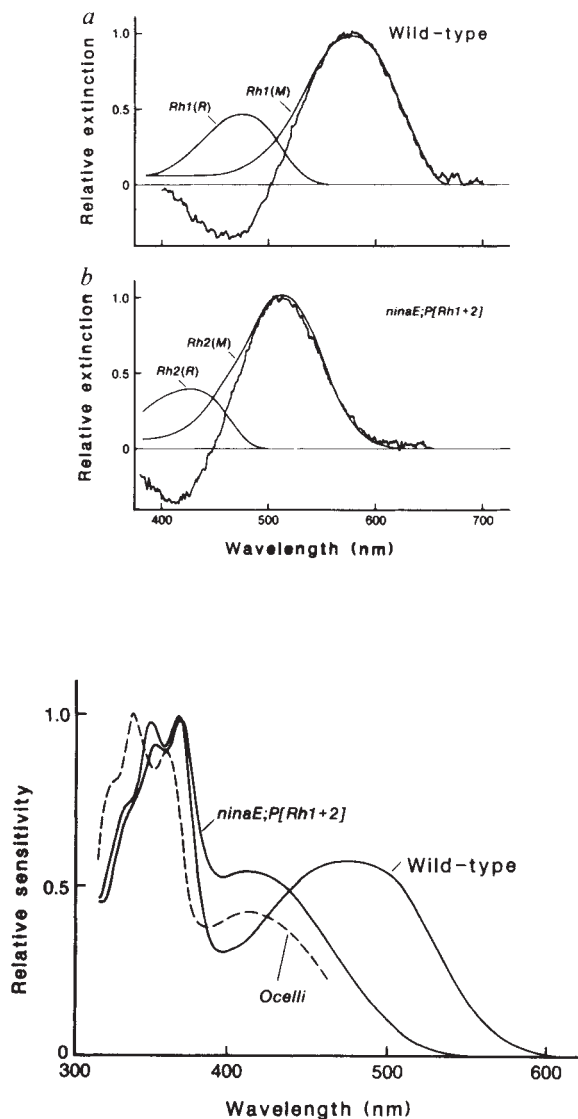


**Fig. 2** Difference spectra derived from microspectrophotometry of the retina of control and *ninaE*;P[*Rh1*+2] flies. All experiments were carried out on white-eyed flies (genetic background *w*<sup>1118</sup>) to eliminate any effects of screening pigments. In this and subsequent figures, for simplicity control flies (*w*<sup>1118</sup>; *ninaE*<sup>+</sup>) are referred to as wild-type. Heads from living animals were cut off, placed in Ringers solution and mounted between coverslips, with eyes directed to the objective of the microspectrophotometer (Leitz MPV II, equipped with Zeiss-ultrafluor optics, combined with Oriel monochromator 7240). Light passing through one of the eyes was selected by the measuring diaphragm. The setup was driven by an IBM AT03 computer via a Data Translation multifunction board DT 2801-A. Difference spectra, experimental details described by Kirschfeld *et al.*<sup>21</sup>, were determined as follows: First, the eye was illuminated with strong light of wavelength  $\lambda_1$ , shifting the equilibrium of the two pigment states (R and M). Afterwards, transmission was measured continuously through the spectrum. The measuring light did not modify the equilibrium between the two pigment states significantly. Thereafter, another strong light of wavelength  $\lambda_2$  was used to shift the pigment into a different equilibrium. Then, the absorption was measured again through the spectrum. The difference spectrum  $\Delta E(\lambda)$  was calculated according to  $\Delta E(\lambda) = [\log(I_{\lambda_1}(\lambda)/I_{\lambda_2}(\lambda))]$ , whereby  $I(\lambda)$  are the intensities transmitted after adaptation to light of  $\lambda_1$  or  $\lambda_2$ , respectively. In the case of wild-type flies,  $\lambda_1$  and  $\lambda_2$  were 584 nm and 456 nm. For *ninaE*;P[*Rh1*+2] transformants,  $\lambda_1$  and  $\lambda_2$  were 524 nm and 402 nm. Relative extinctions, each normalized to 1.0 were plotted against wavelength. The isobestic point (0-crossing) as well as the entire curve for the Rh2 photopigment in *ninaE*;P[*Rh1*+2] flies is shifted about 60 nm to the shorter wavelengths when compared to the Rh1 photopigment of wild-type flies. The inset shows that host *ninaE* flies do not display any appreciable difference spectrum. The *ninaE*;P[*Rh1*+2] curve in the inset is replotted simply to show the relative scale of the two curves.

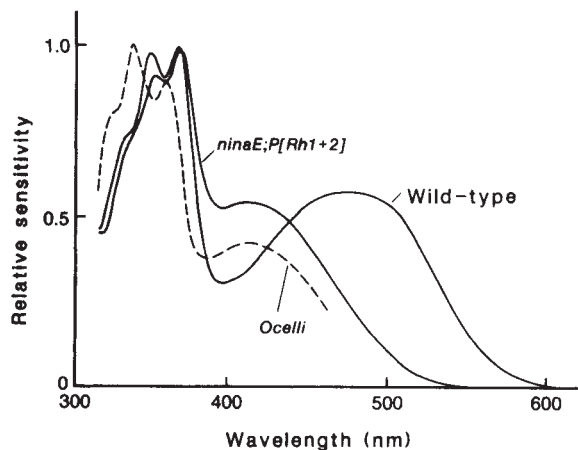
In addition to the sensitivity peak near 480 nm, R1–6 cells expressing Rh1 have a characteristic triplet of sensitivity peaks in the UV region of the spectrum. This is the signature of a previously identified UV-sensitizing pigment, 3-hydroxy-retinol<sup>20,28</sup>, whose function is to extend the range of response of R1–6 cells by absorbing photons in the UV and transferring the energy to the visual pigment molecule. Interestingly, R1–6 cells expressing Rh2 also display this triplet of UV sensitivity peaks (Fig. 4). Thus, the sensitizing pigment normally produced by the R1–6 cells is capable of efficiently sensitizing Rh2 opsin. The slight shift to the shorter wavelengths of the UV triplet in ocellar versus R1–6 photoreceptors may indicate that the sensitizing pigment differs in the two cell types.

Normally, R1–6 cells, like many invertebrate photoreceptors, undergo a prolonged depolarizing afterpotential (PDA) that persists after the cessation of the stimulus whenever a substantial amount of rhodopsin (R) is converted to the dark stable meta-rhodopsin (M)<sup>16,18</sup>. Its physiological basis may be the result of the continued activity of an internal transmitter in the photo-transduction cascade<sup>30</sup>. The PDA can be suppressed by photo-converting M back to R. Figure 5 shows that R1–6 cells from





**Fig. 3** The spectra of the R and M forms of the Rh1 (a) and Rh2 (b) photopigments. To determine the relative extinction spectra of the two pigment states (R and M), we fitted the difference spectra by shifting pigment absorptions on a  $\lambda^{1/4}$  scale (see Barlow<sup>43</sup>). The fit was done using the Gauss-Newton curve fitting option of ASYSTANT (Macmillan Software, New York). As is typical with invertebrate pigments the M form has a higher extinction coefficient and absorbs at longer wavelengths than the corresponding R form. From these data we conclude that the peak sensitivity of the Rh2 photopigment is near 420 nm and that it interconverts to a M form which absorbs maximally at approximately 520 nm. Rh (R) and Rh (M) stand for photopigment R and M form, respectively.



**Fig. 4** Spectral sensitivity of control and transformed *ninaE;P[Rh1+2]* flies. The method applied is based on the 'light-clamp' technique of Franceschini<sup>29</sup>. A quartz neutral density wedge (optical density 0–4) is rotated in the path of the stimulating light in such a way that the electroretinogram (ERG) is constant during the scan through the spectrum. To get high resolution spectra (1-nm bandpass) a double monochromator (Zeiss MM12) was used. This was crucial for resolving the vibrational fine structure of the sensitizing pigment. To increase the signal-to-noise ratio of the photoresponse, we chopped the stimulus light (5–20 Hz) as a.c.-signals of photoreceptors are more stable than d.c.-signals. The amplitude of the ERG was determined by digitally integrating over the area of the a.c.-signal. The triplet of sensitivity peaks in the UV is evident in both wild-type and transformed flies, implying that the sensitizing pigment (3-OH-retinal)<sup>20,28</sup> works to augment the UV sensitivity of the Rh1 or the Rh2 photopigment when either is expressed in R1–6 photoreceptors. The main broad peak of Rh2 sensitivity, near 420 nm, correlates well with our microspectrophotometric analysis of the R form of the Rh2 photopigment. The wild-type sensitivity profile shows a main peak near 480 nm, also in accord with the microspectrophotometric analysis of the R form of Rh1. The dotted curve shows the sensitivity profile of the ocelli of *Calliphora*<sup>31</sup>. Note that there is a good match in spectral profile between this ocellar and the Rh2 photopigment of *Drosophila*.

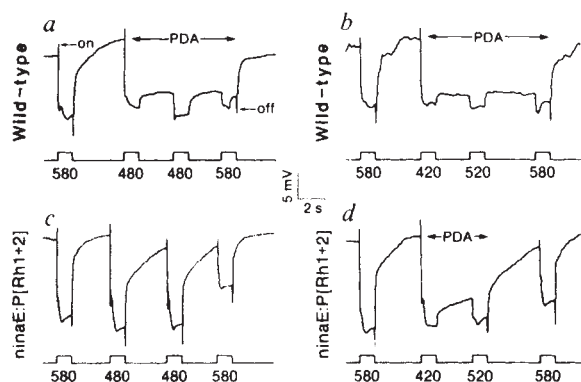
wild-type flies (panel a) undergo a PDA when illuminated with strong blue (480-nm) light. As expected, these cells are refractory to subsequent PDA inducing stimuli. The remaining response is due to activity in R7 and R8 cells<sup>16</sup>. Strong orange light (580 nm), which converts M back to R, suppresses the PDA and brings the cells back to their resting or dark-adapted state. Figure 5 (panel c) shows that *ninaE;P[Rh1+2]* flies do not undergo a PDA when illuminated with the same light stimuli. This, however, is not surprising because 480-nm light is closer to the M peak than the R peak of the Rh2 photopigment (see Fig. 3). To ascertain whether R1–6 cells expressing Rh2 opsin are capable of supporting a PDA, we tested both 420-nm and 520-nm light as they represent the maxima of the R and M forms of the Rh2 visual pigment (Fig. 3). Indeed, Fig. 5 (panel d) demonstrates that strong 420-nm light triggers a PDA which can be suppressed specifically by strong 520-nm light. In control flies (panel b), both 420-nm and 480-nm bleaching lights lead to a PDA, but unlike in *ninaE;P[Rh1+2]* flies 520-nm light is incapable of converting a sufficient amount of M back to R to successfully suppress the PDA. Thus, it is clear that Rh2 is capable of generating a PDA in the novel environment of the R1–6 cells but that the wavelengths needed to elicit and suppress the PDA are specifically tuned to the R and M forms of the

Rh2 opsin. The finding that Rh2 triggers a PDA when in R1–6 cells, which requires a substantial amount of R to M conversion<sup>30</sup>, demonstrates that most Rh2 pigment molecules are active and contribute to transduction in these flies.

### Rh2 is an ocellar photopigment

Both the microspectrophotometric and the electroretinogram data suggested that Rh2 displays a spectral sensitivity which is very similar to the ocellar photopigment described for *Musca*<sup>31</sup>, *Calliphora*<sup>31</sup> and *Drosophila*<sup>19</sup>. The reported spectral sensitivities of R8 cells studied in these same species are shifted to the red compared to R1–6 (refs 11, 22, 23). In contrast, the Rh2 photopigment shows a sensitivity peak which is shifted significantly to the blue region of the spectrum and has, unlike the R8 cell spectral sensitivity, a pronounced contribution by a UV-sensitizing pigment just as the ocelli<sup>31</sup> (Figs 3 and 4). Therefore, we entertained the possibility that Rh2 is an ocellar photopigment and not a major R8 photopigment.

To examine this possibility we studied the expression of Rh2 in *Drosophila* lines that lack particular photoreceptor cell types. We extracted poly(A)<sup>+</sup> RNA from the heads of normal flies and the heads of mutants that included *eyes absent*<sup>32</sup> (lacks eyes only), *no-ocelli*<sup>33</sup> (lacks ocelli only), and *sine oculis*<sup>34</sup> (lacks eyes

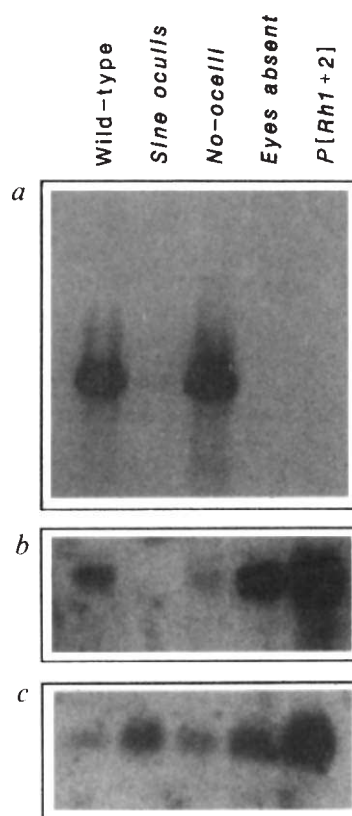


**Fig. 5** Prolonged depolarizing afterpotentials (PDAs) in wild-type and *ninaE*; *P[Rh1+2]* flies. Electrophysiological traces recorded with a wick electrode on the surface of the eye were measured during and after brief stimulations of strong light ( $5 \times 10^{16}$  photons  $\text{cm}^{-2} \text{s}^{-1}$ ) of the indicated wavelengths. Panel *a* shows that intense 580-nm light elicits a maximal receptor potential but no PDA in wild-type flies. Stimulation with 480 nm (the absorption maximum of Rh1(R)), leads to a PDA in control but not in *ninaE*; *P[Rh1+2]* flies (panel *b*). Further 480-nm stimulation of control flies leads to only a small response primarily from R7 and R8 (ref. 16). Finally, bright 580-nm light (absorption maximum of Rh1(M)), terminates the PDA and resensitizes the R1–6 photoreceptors. Panel *d* shows that it is possible to elicit a PDA in *ninaE*; *P[Rh1+2]* flies but that different wavelengths of stimulating light are necessary. Intense 420-nm light (absorption maximum of Rh2(R)), selectively converts R to M in both Rh1 and Rh2 and leads to a PDA in both specimens. This PDA in *ninaE*; *P[Rh1+2]* flies is selectively terminated by strong 520-nm light (absorption maximum of Rh2(M)). In contrast, 580-nm light is required to terminate the PDA of control flies. Lower traces indicate the location and duration of light stimulation. Numbers refer to wavelength ( $\pm 10$  nm).

and ocelli). Figure 6 shows RNA blots hybridized with probes specific for the *Rh1* (panel *a*) and *Rh2* (panel *b*) genes. As can be seen, *Rh2* transcript is present in fly heads containing ocelli, whether or not they have compound eyes (wild-type and *eyes absent*). This is in contrast to the expression profile for the *Rh1* opsin, where transcript is only seen in fly heads containing intact retinas whether or not they have ocelli (wild-type and *no-ocelli*). These results demonstrate that most *Rh2* expression is restricted to the ocelli. Direct observation of the sites of expression of the *Rh2* gene by *in situ* hybridization to tissue sections are in agreement with this result by showing that *Rh2* is abundantly expressed in the ocelli<sup>25</sup>. Similar results have recently been obtained by analysing the site of expression of a *Rh2*- $\beta$ -galactosidase fusion protein<sup>26</sup>. Taken together, these results conclusively demonstrate that *Rh2* corresponds to the ocellar visual pigment of *Drosophila*.

### Optomotor behaviour of *ninaE*; *P[Rh1+2]* flies

R1–6 cells in *Drosophila* share a common photopigment, send axons that synapse in the first optic ganglion, the lamina, and drive optomotor behaviour<sup>12</sup>. R7 and R8 cells have different photopigments, synapse in the second optic ganglion, the medulla, and do not drive optomotor behaviour<sup>13</sup>. To determine whether the *Rh2* opsin is capable of mediating optomotor behaviour through R1–6 cells, we examined optomotor responses in white-eyed control and *ninaE*; *P[Rh1+2]* flies. Test flies were put into a *Drosophila* watch glass surrounded by a concentric cylinder carrying a periodic grating of spatial wavelength 90°. The cylinder could be rotated in both directions eliciting movement stimuli of opposite sign. Normal flies follow the direction of motion and generate walking traces which reflect the direction of the moving stimulus (Fig. 8*a*). Mutant *ninaE*

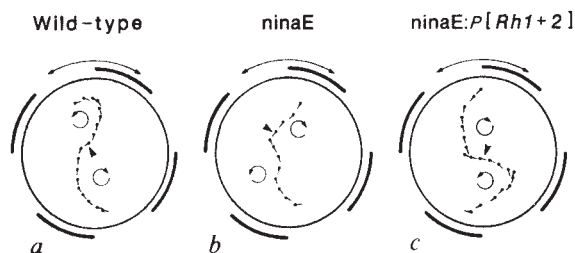


**Fig. 6** *Rh2* is an ocellar photopigment. Poly(A)<sup>+</sup> RNAs were extracted from adult heads of wild-type (3  $\mu\text{g}$  per lane), *sine ocelli* (6  $\mu\text{g}$  per lane), *no-ocelli* (3  $\mu\text{g}$  per lane) and *eyes absent* (6  $\mu\text{g}$  per lane) mutant flies as described by Zuker *et al.*<sup>5</sup>. Total poly(A)<sup>+</sup> RNA was also isolated from *ninaE* flies transformed with the [*Rh1+2*] chimaeric gene. The RNAs were gel-fractionated, blotted and hybridized to probes specific for the *Rh1* (panel *a*) or the *Rh2* (panel *b*) genes. The RNA blot shown in panel *c* was hybridized to a radiolabelled probe encoding the ribosomal protein rp49 (ref. 44) to control for the integrity of the RNAs. *Rh1* transcript is expressed at normal levels in the heads of flies containing a full complement of R1–6 photoreceptors (wild-type and *no-ocelli*) but is dramatically decreased (*sine ocelli*) or absent (*eyes absent*) in flies lacking R1–6 cells. The small amount of transcript seen in *sine ocelli* reflects the incomplete penetrance of the mutation. Panel *b* demonstrates that *Rh2* transcript is present at normal levels in heads containing ocelli whether or not they contain retinas (wild-type and *eyes absent*). *Rh2* transcript, however, is absent or severely reduced in flies lacking ocelli irrespective of the presence of retinas (*sine ocelli* and *no-ocelli*).

flies do not respond to the motion of the striped pattern (Fig. 8*b*). This is expected as all 'optomotor input' is mediated through the R1–6 cells<sup>13</sup> and these flies lack all visual pigment in these cells<sup>35</sup>. But, when expressing the *Rh2* photopigment in their R1–6 cells, these same host flies clearly regain optomotor response. Therefore, the R1–6 photoreceptors of *ninaE*; *P[Rh1+2]* flies, though they express a visual pigment of a different class of cell, must still make the central connections necessary to mediate an optomotor response. It is interesting that in larger male flies R7 and R8 cells at the anterior margin of the retina appear to have the same visual pigment as is present in R1–6 cells<sup>24,36</sup>. These cells do not, like normal R7 and R8, send axons to the medulla, but like R1–6, they project axons to the lamina<sup>37</sup>. This would suggest a direct correlation between photopigment type and central wiring. Our results, however, indicate that the genetic control of the central wiring of a photoreceptor is clearly not dependent on the type of opsin the cell expresses. These two cellular characters must thus be regulated independently.



**Fig. 7** Optomotor behaviour of white-eyed (wild-type) flies (*a*), *ninaE* mutants (*b*), and *ninaE*; *P*[*Rh1+2*] transformants (*c*). A TV camera connected to a video recorder was positioned above the vial monitoring the movements of fly and stimulus. Fifty walking traces of five flies of each genotype were reconstructed frame by frame (1 per 100 ms). Each point represents the position of the fly's head at a given instant and the line the direction of its body. For clarity, only every fifth frame is shown. When the fly was near the middle of the watch glass (arrowhead) the rotation of the striped drum was reversed. Small curved arrows indicate the sense of direction of the drum. Thus in *a* it is clear that this wild-type fly follows the sense of the rotating pattern. In *b* a *ninaE* fly shows no optomotor response. In *c* a transformed *ninaE*; *P*[*Rh1+2*] fly regains normal optomotor behaviour.



## Conclusions

In this article we analyse the spectral properties of the Rh2 photopigment which was first identified solely on the basis of DNA sequence homology with the gene encoding the major *Drosophila* opsin (Rh1)<sup>7</sup>. Cowman *et al.*<sup>7</sup> have previously shown that Rh2 is not expressed in R1-6 or R7 cells but appears to be expressed in the retina at low levels in a subpopulation of R8 photoreceptors. Through a variety of experimental procedures, including microspectrophotometry, electrophysiology and expression studies, we have demonstrated that Rh2 is an ocellar photopigment. The remaining signal of Rh2 in flies without ocelli (Fig. 6b) may reflect the presence of a homologous, cross-hybridizing opsin, or the incomplete penetrance of the *no-ocelli* phenotype (that is, that there are remnants of ocelli in homozygous mutant flies).

Zuker *et al.*<sup>14</sup> have shown that R1-6 cells expressing Rh2 are capable of responding to light. This indicates that there must be common features in the transduction process in the ocellar and R1-6 photoreceptors. This finding is supported by the existence of a number of mutants in *Drosophila* that affect visual function in all photoreceptor cell classes<sup>38</sup>. There are, however, a number of mutants that affect only specific subsets of photoreceptor cells<sup>11,39</sup>, indicating that there must also be features unique to the different cell types. We have shown that the Rh2 opsin when expressed in R1-6 cells can support the production of a PDA, a result that may be particularly interesting because a previous study showed that *Drosophila* ocellar photoreceptors do not undergo a PDA when illuminated with strong light of a variety of wavelengths<sup>19</sup>. Thus the ocellar photoreceptors using the same visual pigment molecule may be incapable of support-

ing a PDA. Alternatively the narrow spectral window of illumination which we found to be necessary to maximally photoconvert Rh2 (R) to Rh2 (M) may account for the inability of the previous study to generate a PDA in the ocelli.

In addition to rescuing phototransduction of host *ninaE* flies, the transformed chimaeric gene leads to the recovery of more central visual functions such as optomotor behaviour. We expect that the action spectrum of the optomotor response of *ninaE*; *P*[*Rh1+2*] flies will reflect the new spectral sensitivity of their R1-6 cells. This opens the possibility of tuning an animal's visual behaviour through site-directed *in vitro* mutagenesis of the visual pigment molecule.

In conclusion, we have shown that it is possible to characterize in detail a minor photopigment by misexpressing it in a well defined photoreceptor cell type. The possibility of functionally dissecting individual components of the phototransduction machinery by isolating and reintroducing the genes of interest into the intact organism under the control cell-type-specific promoters<sup>14,27,40</sup> should provide significant insights into the mechanisms underlying visual function<sup>41,42</sup>.

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# Tolerance in T-cell-receptor transgenic mice involves deletion of nonmature CD4<sup>+</sup>8<sup>+</sup> thymocytes

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*The mechanism of self-tolerance is studied in T-cell-receptor transgenic mice expressing a receptor in many of their T cells for the male (H-Y) antigen in the context of class I H-2D<sup>b</sup> MHC antigens. Autospecific T cells are deleted in male mice. The deletion affects only transgene-expressing cells with a relatively high surface-density of CD8 molecules, including nonmature CD4<sup>+</sup>CD8<sup>+</sup> thymocytes, and is not caused by anti-idiotypic cells.*

T LYMPHOCYTES recognize antigens on the surface of other cells in the context of molecules encoded by the major histocompatibility complex (MHC)<sup>1</sup> by virtue of the heterodimeric T cell receptor (TRC) which is composed of  $\alpha$  and  $\beta$  polypeptide chains<sup>2,3</sup>. In binding to its ligand, the  $\alpha\beta$ TCR is assisted by CD8 or CD4 accessory molecules<sup>4,5</sup>, which presumably interact with nonpolymorphic portions of class I or class II MHC molecules respectively<sup>6-10</sup>. Mature T lymphocytes usually do not respond to self-MHC molecules presenting self-antigens. The question of whether the mechanism of immunological tolerance involved deletion of autospecific lymphocytes has concerned immunologists over decades<sup>11</sup>, but no direct evidence for such a mechanism has been obtained, because the great diversity of receptors generated during lymphocyte development had made it impossible to follow individual clones of cells expressing receptors specific for self-antigens.

Recently, two groups of investigators obtained monoclonal antibodies (mAb) against the products of certain V $\beta$  genes that are expressed with unusually high frequency on T cells specific for certain class II MHC-associated alloantigens<sup>12-14</sup>. Using these antibodies, Kappler *et al.* and MacDonald *et al.* were able to show that in mice expressing the relevant class II MHC-associated antigens, cells expressing the particular V $\beta$  gene

products were absent from the pool of peripheral T cells and medullary thymocytes<sup>12-14</sup>, but were present among cortical CD4<sup>+</sup>8<sup>+</sup> thymocytes<sup>12,13</sup>. These results can be explained by deletion of autospecific cells, but the alternative possibility that their absence is the result of a change of their phenotype caused by modulation or masking of surface molecules has not been excluded.

The development of transgenic mice offers another approach to analyse the mechanism of self-tolerance. To this end we have constructed transgenic mice expressing in a large fraction of their T cells an  $\alpha\beta$ TCR specific for a minor histocompatibility antigen (H-Y) present on male, but not female, cells. Fertilized eggs obtained from a cross of C57BL/6J  $\times$  DBA/2J mice were injected with genomic DNA harbouring the productively rearranged TCR  $\alpha$  and  $\beta$  genes isolated from the B6.2.16 cytolytic T-cell clone<sup>15</sup>. This clone is specific for H-Y antigen in the context of class I (H-2D<sup>b</sup>) MHC antigen and expresses a TCR  $\beta$ -chain coded in part by the V $\beta$ 8.2 gene segment which can be identified by the F23.1 antibody<sup>16</sup>.

The transgenic founder mouse 71 contained four copies of the  $\alpha$  and two copies of the  $\beta$  transgenes integrated on the same chromosome<sup>17</sup>. It was crossed with C57L mice expressing H-2<sup>b</sup> MHC antigens, but lacking the V $\beta$ 8 gene family. Here we show that cells with the phenotype of the B6.2.16 clone that responded to H-Y antigen were frequent in female but not in male transgenic offspring, despite the fact that peripheral T cells in animals

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**Table 1** Frequency of male (H-Y) antigen-specific precursors of proliferating T cells (PT-P) among CD4<sup>+</sup>8<sup>+</sup> and CD4<sup>+</sup>8<sup>+</sup> T cells from normal and  $\alpha\beta$ TCR transgenic mice

| Stimulation:<br>spleen cells<br>(3000R) + IL-2 | CD4/CD8<br>phenotype            | C57L<br>female |                | Donor of responding T cells<br>$\alpha\beta$ TCR transgenic |           |             |     |             |           |
|------------------------------------------------|---------------------------------|----------------|----------------|-------------------------------------------------------------|-----------|-------------|-----|-------------|-----------|
|                                                |                                 | 1/frequency    | $p^*$          | female                                                      |           | male        |     |             |           |
|                                                |                                 |                |                | 1/frequency                                                 | $p$       | 1/frequency | $p$ | 1/frequency | $p$       |
| C57BL/6 female                                 | CD4 <sup>+</sup> 8 <sup>+</sup> | >25,000        |                | >25,000                                                     |           | >25,000     |     | >25,000     |           |
| C57BL/6 female<br>+ Con A                      | CD4 <sup>+</sup> 8 <sup>+</sup> | 2.3            | (1.6-3.3)      | 1.8                                                         | (1.2-2.6) | 0.87        |     | 2.3         | (1.3-4.0) |
|                                                | CD4 <sup>+</sup> 8 <sup>-</sup> | NT†            |                | 6.4                                                         | (4.5-9.1) | 0.60        |     | NT†         |           |
| C57BL/6 male                                   | CD4 <sup>+</sup> 8 <sup>+</sup> | 15,985         | (5,029-50,802) | 6.6                                                         | (4.8-9.0) | 0.67        |     | >25,000     |           |
|                                                | CD4 <sup>+</sup> 8 <sup>-</sup> | NT†            |                | >25,000                                                     |           |             |     | NT†         |           |

Lymph node cells were stained with a mixture of anti CD4-PE and anti-CD8-FITC mabs (see Fig. 1). CD4<sup>+</sup>8<sup>-</sup> and CD4<sup>+</sup>CD8<sup>+</sup> T cells were separated on fluorescein activated cell sorter (FACS440, Becton Dickinson). Limiting numbers of CD4<sup>+</sup>8<sup>-</sup> CD4<sup>+</sup>8<sup>+</sup> T cells (24 wells per group) were cultured for 8 days together with irradiated (3,000R) spleen cells ( $5 \times 10^5$  cells per cell) and interleukin-w (5% v/v) of partially purified supernatant from Con A-stimulated rat spleen cells<sup>24</sup> without or with Con A ( $2.5 \mu\text{g ml}^{-1}$ ). Cells were collected after addition of [<sup>3</sup>H]thymidine for the last 12 h of culture and incorporated radioactivity was measured by liquid scintillation counting. Negative control cultures contained no responder cells. Frequencies were calculated as described elsewhere<sup>25</sup>.

\* Probability,  $p$ , attached to the computed  $\chi^2$  (ref. 25). † NT, not tested.



of both sexes expressed both transgenes<sup>17</sup>. T cells in male (but not female) mice had an abnormal CD4/CD8 phenotype: over 90% of T cells in male transgenic mice were CD4<sup>+</sup>CD8<sup>-</sup>, or expressed only low levels of CD8 molecules, and the numbers of CD4<sup>+</sup>CD8<sup>-</sup> T cells were very small. The cellular composition of male thymuses revealed that this unusual phenotype of peripheral T cells was the consequence of deletion of auto-specific thymocytes expressing high levels of CD8 molecules, predominantly cortical CD4<sup>+</sup>CD8<sup>+</sup> thymocytes. The deletion process spared cells expressing low levels of CD8 molecules, but affected the precursors of single positive CD4<sup>+</sup>CD8<sup>-</sup> cells that were not male-specific. This latter observation provides strong evidence that double-positive CD4<sup>+</sup>CD8<sup>+</sup> thymocytes contain precursors of single positive CD4<sup>+</sup>CD8<sup>-</sup> and CD4<sup>+</sup>CD8<sup>+</sup> T cells.

## T cells in females

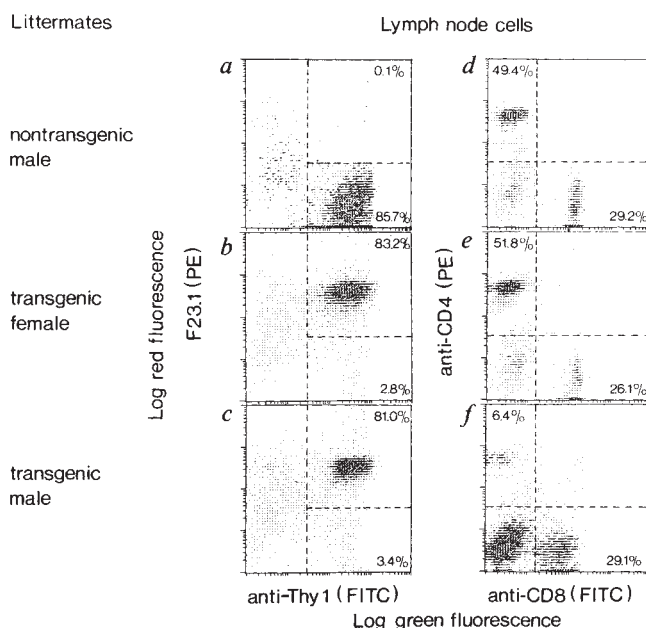
Lymph nodes of female transgenic mice contained normal proportions of CD4<sup>+</sup>CD8<sup>+</sup> and CD4<sup>+</sup>CD8<sup>-</sup> T (Thy1<sup>+</sup>) cells which had normal levels of CD4 as well as CD8 accessory molecules (Fig. 1a, b, d and e). But these differed in two respects from T cells in normal mice. Firstly, as previously described for  $\beta$  transgenic mice<sup>15</sup>, most of them expressed the transgenic  $\beta$  chain on their surface (Fig. 1b). Secondly, as shown by limiting dilution analysis of CD4<sup>+</sup>CD8<sup>+</sup> T cells, one in six proliferated specifically in response to C57BL/6 male stimulator cells, as compared with one in 16,000 in normal C57BL/6 female mice (see Table 1). As only every second plated T cell responded to concanavalin A (Con A), we conclude that at least 30% of CD4<sup>+</sup>CD8<sup>+</sup> T cells in transgenic females have a phenotype similar to that of the B6.2.16 clone. CD4<sup>+</sup>CD8<sup>-</sup> T cells from transgenic mice did not show any male-specific proliferation, but did respond to Con A (Table 1).

## T cells in males

As in transgenic females, lymph nodes of transgenic males contained normal proportions of Thy1<sup>+</sup> cells, and most of them expressed the transgenic  $\beta$  chain on their surface (Fig. 1c). Northern blot analysis of the  $\alpha$  transgene revealed comparable levels of expression in T cells from female and male mice<sup>17</sup>. However, the CD4/CD8 phenotype of T cells in male mice was very different from that of females: 58% of Thy1<sup>+</sup> cells were CD4<sup>+</sup>CD8<sup>-</sup>, 35% were CD4<sup>+</sup>CD8<sup>+</sup> but expressed low levels of CD8, and 7% were CD4<sup>+</sup>CD8<sup>+</sup> and expressed normal amounts of CD4 (Fig. 1f). Limiting dilution analysis showed that one in two CD4<sup>+</sup>CD8<sup>+</sup> T cells could be induced to grow by Con A. There was, however, no detectable response to male C57BL/6 stimulator cells (Table 1). Likewise, CD4<sup>+</sup>CD8<sup>-</sup> and CD4<sup>+</sup>CD8<sup>+</sup> T cells were unresponsive to H-Y antigen (data not shown). These results indicate that male-specific T cells with the phenotype of the B6.2.16 clone are absent from male transgenic mice and that the lack of or low level of CD8 on transgene-expressing cells precluded male-specific responses. This conclusion is supported by the observation that cytolytic activity of the B6.2.16 clone can easily be inhibited by anti-CD8 antibodies (not shown), and by CD8 gene transfection experiments which show that CD8 molecules strongly assist antigen recognition by T cells<sup>4,5</sup>. As shown elsewhere<sup>17</sup>, a high proportion of CD4<sup>+</sup>CD8<sup>-</sup> T cells in male mice expressed both transgenes, but had their endogenous  $\alpha$  and  $\beta$  genes in germline configuration. This result, and the fact that only a few T cells expressed normal amounts of CD8 in male transgenic mice, is consistent with the notion that most T cells in male mice and CD8<sup>+</sup> T cells in female mice carry transgenic  $\alpha\beta$ TCR on their surface. But, owing to reduced density of CD8 molecules in male mice, they are not autoreactive.

## Thymocytes

The number of thymocytes was drastically lower in male ( $0.5\text{--}1.6 \times 10^7$  per thymus) than in female ( $1.0\text{--}1.6 \times 10^8$  per thymus) transgenic mice.



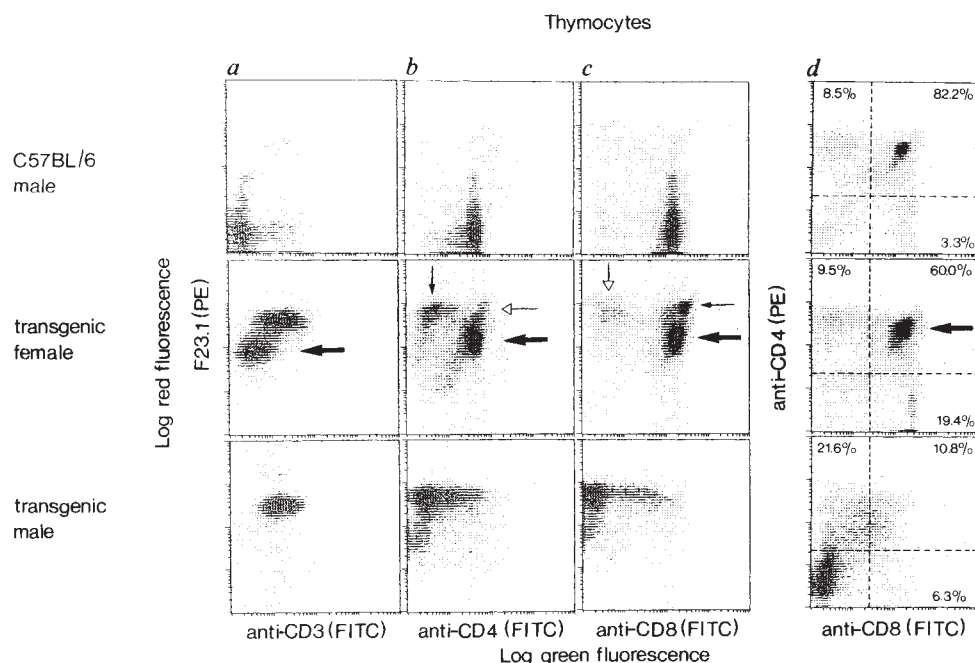
**Fig. 1** Comparison of cell surface expression of F23.1<sup>+</sup> TCR  $\beta$  chain, CD4 and CD8 molecules on lymph node T (Thy1<sup>+</sup>) cells from female and male  $\alpha\beta$ TCR transgenic mice and their nontransgenic male littermate as analysed by two-colour flow cytometry. Lymph node cells were stained with biotinylated F23.1 monoclonal antibody (mAb) followed by a mixture of fluorescein (FITC)-labelled anti-Thy1 mAb with phycoerythrin-streptavidin (PEA) (a, b and c) or with PE-conjugated anti-CD4 mAb followed by FITC conjugated anti-CD8 mAb (d, e and f). In panels a, b and c some Thy1<sup>+</sup> cells (B cells) stain nonspecifically with F23.1 mAb due to the binding by Fc receptor. The presented data were obtained with one pair of 7-week-old  $\alpha\beta$ TCR transgenic female and male littermates. The same results were obtained with 3 other pairs of transgenic mice.

**Methods.** Single cell suspensions were prepared from lymph nodes (mesenteric, axillary, inguinal) and washed twice in RPMI-1640 and once in PBS with 5% FCS. For staining the following mAbs were used: FITC-conjugated anti-Thy1 (ref. 21), biotin-conjugated F23.1 (ref. 16), PE-conjugated anti-CD4 (anti-mouse L3T4, Becton Dickinson) FITC conjugated anti-CD8 (anti-mouse Lyt2, Becton Dickinson). Biotin or FITC conjugation of mAbs was performed by standard procedures. Optimal concentrations of staining reagents were determined in preliminary experiments. All incubations and washings were done at 4°C. Cells ( $0.5\text{--}1 \times 10^6$ ) were incubated with either biotinylated F23.1 mAb (a, b and c) or anti-CD4-PE mAb (d, e and f). After 20 min, cells were washed twice and incubated again for 20 min with anti-Thy1-FITC mAb plus PEA (Becton Dickinson) (a, b and c) or with anti-CD8-FITC mAb (d, e and f). Finally, cells were washed three times in PBS 5% FCS and analysed for two-colour fluorescence on FACScan (Becton Dickinson) flow cytometer with a single Argon laser and logarithmic intensity scales using FACScan research software program (FRSP). Ten thousand viable cells were analysed in each sample. Dead cells were excluded from analysis using a combination of low-angle and sideways light scatter. The results are presented as 'density' plots, generated by analysis of processed data reduced to a  $64 \times 64$  matrix with 16 levels. Percentages of stained and non-stained cells were calculated using FRSP. Markers were set against the 'density' plots of control samples which involved substitution of diluent alone for either one or both antibodies.

As shown in Fig. 2a, double-staining with F23.1 and CD3 antibodies demonstrated that most (>95%) thymocytes from transgenic females and males expressed the  $\beta$  transgene, and that the amount of TCR expression corresponded to the higher values of the normal spectrum of TCR densities observed in C57BL/6 mice.

In the thymus of transgenic females, two populations expressing different levels of TCR could be distinguished (Fig. 2a, middle panel). The one with relatively low TCR density included

**Fig. 2** Expression of F23.1<sup>+</sup> TCR  $\beta$  chain, CD3, CD4, and CD8 molecules on thymocyte subpopulations from normal C57BL/6 and from  $\alpha\beta$ TCR transgenic female and male mice. In panel *a*, cells were incubated consecutively with anti-CD3 mAb, FITC-conjugated goat anti hamster immunoglobulin, mouse immunoglobulin, biotinylated F23.1 mAb and PEA. In panels *b* and *c*, cells were stained both biotinylated F23.1 mAb, followed by a mixture of anti-CD4-FITC (*b*) or anti-CD8-FITC (*c*) mAbs with PEA. In panel *d*, cells were stained with anti-CD4-PE followed by anti-CD8-FITC mabs. The number of cells per thymus in C57BL/6 male,  $\alpha\beta$ TCR transgenic female and  $\alpha\beta$ TCR transgenic male were:  $100 \times 10^6$ ,  $105 \times 10^6$  and  $13 \times 10^6$  respectively. Thick arrows indicate the population of CD4<sup>+</sup>8<sup>+</sup> thymocytes expressing a lower level of TCR, which is mostly depleted in male thymus. Open-head arrows indicate the population of CD4<sup>+</sup>8<sup>-</sup>, and thin arrows of CD4<sup>+</sup>8<sup>+</sup>



female thymocytes that express higher levels of TCR. Populations indicated by open-head thin arrows and in the upper left quadrant of middle panels *b* and *c* also contain CD4<sup>+</sup>CD8<sup>-</sup> thymocytes, as indicated by virtual absence of cells in the lower left quadrant of middle panel *c*. Presence of cells in lower left quadrant of middle panel *b* is due to imperfect staining of this particular sample in this experiment. In other experiments, no F23.1<sup>+</sup>CD4<sup>-</sup> cells could be seen under the same conditions.

**Methods.** Single thymocyte suspensions were prepared by squeezing the whole thymus through a nylon mesh into medium RPMI-1640 with 5% FCS. After washing, cells were resuspended in PBS with 5% FCS, counted and stained as indicated above with extensive washings between each step (see Fig. 1). For staining with anti-CD3, unconjugated mAb 145.2c11 (ref. 22) was used. To saturate free binding-sites of second-step reagent, cells were incubated with mouse immunoglobulin (Sigma, 1 mg ml<sup>-1</sup>) for 15 min. FITC-conjugated anti-CD4 (GK1.5, ref. 23) mAb was prepared by standard procedures. Control samples were stained with each reagent alone, or in combinations omitting each single reagent. Ten thousand viable cells were analysed in each sample by FACScan flow cytometry. For details see Fig. 1.

CD4<sup>+</sup>8<sup>+</sup> cells, whereas the other, expressing about tenfold more TCR, contained CD4<sup>+</sup>8<sup>-</sup>, CD4<sup>+</sup>8<sup>+</sup> and CD4<sup>+</sup>8<sup>-</sup> cells (Fig. 2*b* and *c*, middle panels).

Double-staining with CD4 and CD8 antibodies revealed significant differences between transgenic and normal C57BL/6 females with regard to the size of CD4<sup>+</sup>8<sup>-</sup>, CD4<sup>+</sup>8<sup>+</sup> and CD4<sup>+</sup>8<sup>-</sup> thymocyte subpopulations (Fig. 2*d*, upper and middle panels). The proportion of CD4<sup>+</sup>8<sup>-</sup> thymocytes in transgenic females was normal, but the proportion of CD4<sup>+</sup>8<sup>+</sup> thymocytes was enlarged, resulting in a reversed ratio of CD4<sup>+</sup>8<sup>-</sup> to CD4<sup>+</sup>8<sup>+</sup> cells as compared with normal nontransgenic mice. The proportion of CD4<sup>+</sup>8<sup>-</sup> thymocytes was also noticeably higher in transgenic females than in normal mice. The increase in proportion of CD4<sup>+</sup>8<sup>+</sup> and CD4<sup>+</sup>8<sup>-</sup> cells was matched by a corresponding decrease in the size of the CD4<sup>+</sup>8<sup>+</sup> population.

In contrast to the females, the thymus of transgenic males was severely depleted of CD4<sup>+</sup>8<sup>+</sup> cells with decreased expression of TCR, but contained about the same total number of CD4<sup>+</sup>8<sup>-</sup> cells, which constituted the bulk of the population of male thymocytes (Fig. 2*d*, middle and lower panels). Most CD4<sup>+</sup>8<sup>+</sup> cells showed low expression of CD8. Thus, the male thymus was depleted of transgene-expressing cells with relatively high levels of CD4/CD8 accessory molecules and the nonmature CD4<sup>+</sup>8<sup>+</sup> thymocytes expressing decreased amounts of TCR were the main target of depletion.

Because CD4<sup>+</sup>8<sup>+</sup> thymocytes are extremely steroid-sensitive, it was important to find out whether the deletion of these cells in transgenic males was a result of stress rather than of an antigen-specific deletion process. Stress in the male mice could possibly be caused by autoimmunity not detectable by *in vitro* assay. We addressed this question in reconstitution experiments using haemopoietic stem cells from transgenic (F23.1<sup>+</sup>) and nontransgenic C57L (F23.1<sup>-</sup>) mice (Fig. 3). T-cell-depleted

bone marrow cells (BMC) from transgenic females were transferred either along or together with BMC from normal C57L females into lethally X-irradiated female and male C57L recipients. Five weeks after the transfer of the transgenic BMC, the cellular composition of the thymus in male recipients was very much like that in male transgenic mice (Fig. 3*a*, lower panel). But in the thymus of male recipients which had received a mixture of BMC from transgenic and C57L females, CD4<sup>+</sup>8<sup>+</sup> thymocytes derived from F23.1<sup>-</sup> C57L donors developed normally and outgrew the transgenic F23.1<sup>+</sup> cells, which were mostly deleted (Fig. 3*b* and *c*, lower panel). On the other hand, in the female recipient, CD4<sup>+</sup>8<sup>+</sup> thymocytes developed from both F23.1<sup>+</sup> and F23.1<sup>-</sup> donors (Fig. 3*b* and *c*, upper panel). Thus, because the deletion selectively affected transgene-expressing F23.1<sup>+</sup> CD4<sup>+</sup>8<sup>+</sup> thymocytes in the male recipients, this experiment indicates that the deletion is a result of the interaction of autospecific thymocytes with radioresistant male cells in the thymus, and not of stress and steroid release. If the latter possibility were true, the F23.1<sup>-</sup> CD4<sup>+</sup>8<sup>+</sup> thymocytes derived from C57L donors of BMC should also have been affected.

## Discussion

Our study with  $\alpha\beta$ TCR transgenic mice provides new observations relevant to the understanding of the mechanism of self-tolerance and relevant to the clarification of the function of cortical CD4<sup>+</sup>8<sup>+</sup> thymocytes in T-cell development. The drastically decreased number of thymocytes in male but not female mice is direct evidence for a deletion of autospecific cells at the level of CD4<sup>+</sup>8<sup>+</sup> nonmature thymocytes. Furthermore, our results indicate that CD4 and CD8 accessory molecules are involved in the deletion process of autospecific cells.

Two questions relating to the function of double-positive



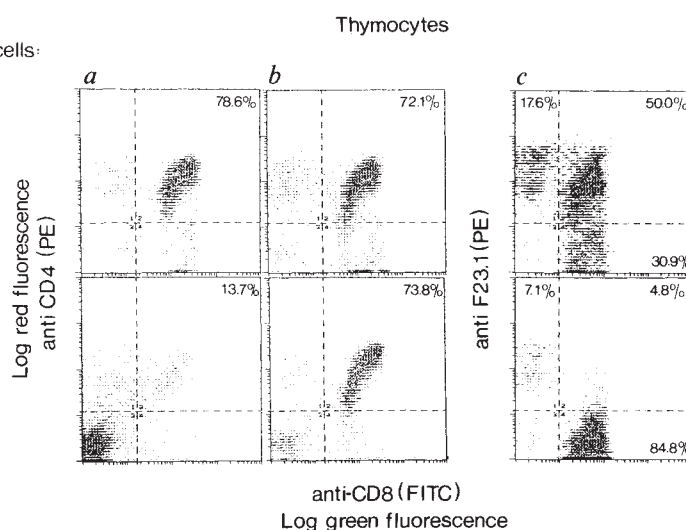
**Fig. 3** Surface phenotype of thymocytes from C57L male and female irradiation chimeras reconstituted with bone marrow of  $\alpha\beta$ TCR transgenic female, either alone (a) or with normal bone marrow from C57L female (b, c). Thymocytes were stained with anti-CD4-PE (a, b) or biotinylated F23.1 (c) mAbs, followed by anti-CD8-FITC mAb (a, b) or a mixture of CD8-FITC mAb with PEA (c). In (a), 80% of thymocytes in the female recipient and 67% in the male recipient were stained with F23.1 mAb (data not shown).

**Methods.** Bone-marrow cells from transgenic or normal C57L donor were treated with cytotoxic anti Thyl mAb (T24, ref. 21) plus rabbit complement (Cedar Lane, Ontario, Canada) for 45 min at 37 °C. After washing,  $5 \times 10^6$  viable cells from the transgenic donor were injected intravenously (i.v.) into lethally irradiated (850R) eight-week-old C57L females and males, either alone or together with  $0.5 \times 10^6$  viable bone marrow cells from normal female C57L. Five weeks later the mice were killed, their thymuses removed and single-cell suspensions prepared, counted and stained with anti-CD4, -CD8 and -F23.1 mAb, and analysed as described in Figs 1 and 2.

Recipient (850R)  
of bone marrow cells:

C57L female

C57L male



CD4<sup>+</sup>8<sup>+</sup> thymocytes are why so many of these cells should die within the thymus<sup>18</sup> and whether or not they contain precursors of single positive CD4<sup>+</sup>8<sup>+</sup> and CD4<sup>+</sup>8<sup>+</sup> cells<sup>19</sup>. Our results show that the death of at least some cortical thymocytes can result from antigen-specific elimination of autoreactive cells. The deletion of nonfunctional, antigen-specific CD4<sup>+</sup>8<sup>+</sup> thymocytes would make sense if CD4<sup>+</sup>8<sup>+</sup> thymocytes contained precursors of functional CD4<sup>+</sup>8<sup>+</sup> and CD4<sup>+</sup>8<sup>+</sup> cells. Consistent with this view is our observation that CD4<sup>+</sup>8<sup>+</sup> cells were severely depleted in male transgenic mice, despite the fact that such cells from transgenic female mice cannot be activated by male cells. An analogous finding has been reported by MacDonald *et al.*<sup>14</sup>, who observed that CD4<sup>+</sup>8<sup>+</sup> and CD4<sup>+</sup>8<sup>+</sup> T cells staining with V $\beta$ 6 antibodies were reduced to the same extent in animals positive for the *Mls*<sup>a</sup>-allele of the minor lymphocyte stimulating locus (*Mls*), even though CD4<sup>+</sup>8<sup>+</sup> from *Mls*<sup>a</sup>-negative animals lacked specificity for *Mls*<sup>a</sup>.

We thus favour the view that at least some double-positive CD4<sup>+</sup>8<sup>+</sup> thymocytes act as precursors for functional single positive cells<sup>20</sup>, even though further investigation is needed. Although we have shown that the deletion predominantly affects CD4<sup>+</sup>8<sup>+</sup> thymocytes, we could argue that it might occur independently of accessory molecules at any stage of T-cell development. But this view is not compatible with our observation that the deletion process spares T cells that lack accessory molecules, or even T cells having low expression of CD8. Thus our experiments provide the first direct evidence that these molecules play a crucial role in the induction of tolerance. Taken together, the three observations made in male transgenic mice, namely the drastically reduced number of CD4<sup>+</sup>8<sup>+</sup> thymocytes, the reduction of CD4<sup>+</sup>8<sup>+</sup> cells and the occurrence of transgene-expressing cells with virtually no CD8, argue that the deletion of auto-specific cells is dependent on CD4 and CD8 accessory molecules.

The results of our experiments with transgenic mice differ in at least two important aspects from others recently reported<sup>12-14</sup> for normal mice. Firstly, in the experiments of Kappler *et al.*<sup>12,13</sup>, the depletion of autospecific T cells did not appear to affect CD4<sup>+</sup>8<sup>+</sup> thymocytes. One possible reason for the difference is that different antigens are under investigation: we are looking at an antigen in the context of class I MHC antigens found throughout the cortex whereas Kappler *et al.*<sup>12,13</sup> are looking at an entity<sup>21</sup> related to class II MHC antigens which are usually not detected in the outer cortex. Thus in the latter case CD4<sup>+</sup>8<sup>+</sup> cells can meet antigen only when reaching the cortico-medullary junction. Consequently only a minor subset of CD4<sup>+</sup>8<sup>+</sup> cells would be deleted in the experiments of Kappler *et al.*, and this

would be difficult to detect. Another possible reason for the different findings is the fact that in our transgenic mice the expression of TCR proteins is skewed towards higher levels of the range observed in CD4<sup>+</sup>8<sup>+</sup> from normal mice. This phenomenon, as well as the increased proportion of CD4<sup>+</sup>8<sup>+</sup> thymocytes in transgenic females, could reflect a positive selection of thymocytes by H-2<sup>b</sup> antigens, or alternatively may be a direct consequence of expression of transgenes. We could argue therefore that the deletion of CD4<sup>+</sup>8<sup>+</sup> thymocytes was easily detected because the majority of CD4<sup>+</sup>8<sup>+</sup> thymocytes in transgenic mice represent a minor and more mature population of CD4<sup>+</sup>8<sup>+</sup> thymocytes that may escape detection in normal mice, especially when representing only a fraction of cells expressing a certain idotype. Whatever the reason for the apparent discrepancy in the results, our data indicate that the nonmature CD4<sup>+</sup>8<sup>+</sup> population can be a target of deletion, whereas it is not clear whether CD4<sup>+</sup>8<sup>+</sup> or CD4<sup>+</sup>8<sup>+</sup> cells are susceptible to the same deletion process.

The second difference between our results and those of Kappler *et al.*<sup>12,13</sup> and MacDonald *et al.*<sup>14</sup> is that these authors did not report the presence of cells with few or no accessory molecules, spared by the deletion. Again in this case, such cells would constitute a very minor population in their experimental system, because the pool of T cells in normal mice can be easily replenished by T cells expressing different TRCs, which is not the case in transgenic mice.

As we observed normal numbers of T cells in the periphery, but not in the thymus, of transgenic males, we propose that the number of peripheral T cells can be adjusted independently of the export of newly formed cells from the thymus. This would allow the accumulation of cells with rare phenotypes in the periphery of male mice, as shown here and in the accompanying paper<sup>17</sup>. The fact that transgene-expressing cells with few or no accessory molecules accumulate in male mice, tends to rule out a role of anti-idiotypic cells in the deletion process; such a mechanism should eliminate transgene-expressing cells, rather than cells expressing high levels of accessory molecules.

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## LETTERS TO NATURE

### Spin-down of the X-ray pulsar GX1+4 during an extended low state

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X-ray pulsars<sup>1,2</sup> are magnetized, spinning neutron stars accreting matter from their binary companions. Their pulse periods  $P$ , ranging over four orders of magnitude, increase and decrease in complex ways<sup>1,3,4</sup>. The more luminous ones tend to show faster spin-up<sup>1,5</sup>. A puzzle is that the spin-up timescales of many X-ray pulsars are much shorter than their binary-evolution timescales, thus apparently violating the steady-state condition. It has therefore been suspected<sup>6</sup> that there exist many 'turned-off' X-ray pulsars currently spinning down undetected. An excellent test for this hypothesis became available using the X-ray pulsar GX1+4, which used to show the fastest spin-up over a decade<sup>1,7-10</sup> and then faded away<sup>11</sup>. Using the X-ray satellite Ginga<sup>12</sup>, we detected GX1+4 at  $\sim 1/40$  the previous intensity, and found that it now has an average spin-down trend. This discovery apparently supports the above hypothesis.

GX1+4 (4U1728-24) is a luminous binary X-ray pulsar near the Galactic centre<sup>13</sup>, with a  $\sim 2$  min (or possibly twice this) pulse period and probably a  $>100$ -day binary period<sup>7,13</sup>. From its discovery in the early 1970s until 1980, it was spinning up rapidly, with  $\dot{P} = -7.5 \times 10^{-8} \text{ s s}^{-1}$  ( $\dot{P}/P = -0.02 \text{ yr}^{-1}$ ) (refs 7-10), the fastest among binary X-ray pulsars<sup>1</sup>. The optical companion is an M6III symbiotic star<sup>13</sup>; it is thus a rather rare system composed of an evolved late-type primary star and a strongly magnetized neutron star. Although in the 1970s GX1+4 had an X-ray intensity of 50-200 mCrab ( $10^{37-38} \text{ erg s}^{-1}$ ; ref. 7), in 1983 it was found by Exosat to be  $<4$  mCrab (ref. 11). This and subsequent Exosat observations (K. Mukai, personal communication) showed that GX1+4 had entered an extended low state ( $<0.5$  mCrab), although the hard X-ray flux<sup>14</sup> might have remained relatively unchanged<sup>15</sup> and the optical emission showed a complex time variation<sup>16,17</sup>.

The present observation was carried out with the large area counter (LAC; M.J.L.T. *et al.*, manuscript in preparation) on

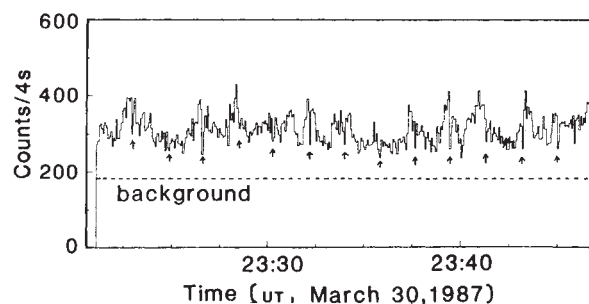


Fig. 1 A part of the 1-20 keV count rate record during the present GX1+4 observation, binned at 4.0 s. The arrows indicate the expected dip recurrence at 110.2 s. The background level, determined using the standard Ginga background estimation procedure (M.J.L.T. *et al.* 1988, manuscript in preparation), should be accurate to  $\sim 2 \text{ cs}^{-1}$ .

board Ginga launched on 5 February 1987<sup>12</sup>. The LAC experiment, a UK-Japan collaboration, uses eight identical proportional counters, achieving  $4,000 \text{ cm}^2$  total effective area. The LAC was pointed to GX1+4 from 07:24 to 09:08 UT on 28 March, and 16:52 on 30 March to 03:02 on 31 March 1987. The available data consisted of 10 separate segments of  $\sim 20$  min each. The data covered an energy range of 1-36 keV with 48 spectral channels and 0.5-s time resolution. Figure 1 shows a portion of the raw X-ray count-rate record; the source was unambiguously detected at about  $33 \text{ cs}^{-1}$  (1-30 keV), or 3 mCrab, and showed significant flickering. The pulse-phase averaged X-ray spectrum is slightly softer than those observed previously<sup>2,8,18</sup>. At a 10 kpc distance, the observed 2-20 keV luminosity becomes  $1.5 \times 10^{36} \text{ ergs s}^{-1}$ .

A standard Fourier analysis revealed a highly significant period at  $\sim 110$  s, together with several higher harmonics, but no other periodicity. Through a folding analysis, we refined the heliocentric period to  $P = 110.233 \pm 0.003 \text{ s}$  (ref. 19) with a reduced chi-squared of 15.4 for 99 degrees of freedom. As plotted in Fig. 2, this value for  $P$  is longer than the value  $109.668 \pm 0.002 \text{ s}$  last obtained in 1980<sup>8</sup>. Therefore the average behaviour of GX1+4 between 1980 and 1987 proves to be spin-down at a rate  $\dot{P} = 2.6 \times 10^{-9} \text{ s s}^{-1}$ , in contrast to the previous rapid spin-up. A joint Australia-Germany-Italy balloon experiment in November 1986 obtained a similar value for  $P$  (A. B. Giles, J. Greenhill and D. P. Sharma, personal communication).

The folded pulse profile (Fig. 3) turned out to be extremely peculiar: the pulse peak involves a sharp dip, which is resolved to two roughly symmetric minima separated by a local narrow maximum. On closer inspection, we can identify individual dips in the raw data of Fig. 1. Such a peculiar feature has never been



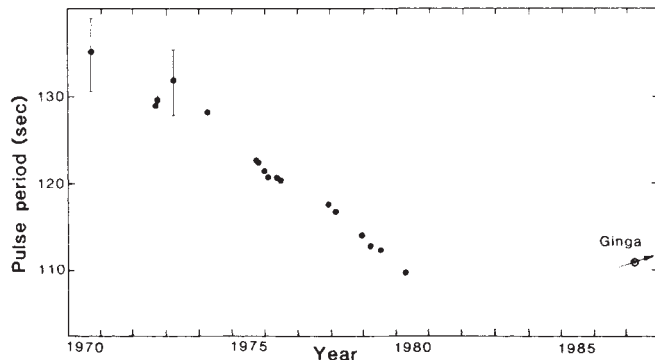


Fig. 2 The pulse-period history of GX1+4. Previous measurements were taken from ref. 9. An inclined arrow superimposed on the Ginga data point indicates the measured instantaneous period-change rate.

observed before either in GX1+4 or in any other X-ray pulsar, although the pulse profile of GX1+4 changed on various time-scales<sup>2,7-10,14,18</sup>. A dramatic change thus took place not only in the pulse period and the X-ray intensity, but also in the pulse profile. Although the causal relation among these events is yet to be investigated, neutron star precession<sup>20</sup> might give some explanation. The dip cannot be due to absorption by cold matter as its depth is rather independent of X-ray energy. Also the dip was found to be quite stable in pulsation phase. The dip may be related to anisotropic X-ray transfer effects in the strong surface magnetic field of the pulsar<sup>21</sup>.

To study possible pulse-period changes during the observation, we folded each of the ten data segments separately at 110.233 s. Using the dip as an ideal phase marker, we then determined the heliocentric pulse arrival time for each data segment. The arrival times thus determined require a positive value of  $P$ ; they are best represented by a combination of  $P = 110.233 \pm 0.003$  s at an epoch of JD 2,446,885.5 (UT 00:00, 31 March 1987) and  $\dot{P} = (3 \pm 1) \times 10^{-8} \text{ s s}^{-1}$  (90% confidence limits). As this value of  $\dot{P}$  is much larger than is explained by the orbital Doppler effect, we believe that GX1+4 was actually spinning down during our observation. This value of  $\dot{P}$  is larger by an order of magnitude than the average value between 1980 and 1987, and surprisingly it amounts to about 40% of the absolute value of  $\dot{P}$  during the regular spin-up (Fig. 2).

Considering that the spin-up of X-ray pulsars is caused by accretion torque<sup>1,5</sup>, the extended low state and the associated spin-down of GX1+4 strongly suggest a real decrease in the mass accretion rate,  $\dot{M}$ . This decrease may be caused by a small change in the structure of the outer envelope of the evolved companion. To explain the observed spin-down, the magnetic braking effect could be invoked<sup>5,22,28</sup>, a fast pulsar can transfer its angular momentum to the accreting matter, when a reduction in  $\dot{M}$  makes the Alfvén radius close to the corotation radius. This mechanism may indeed account for the somewhat similar behaviour of the rapidly rotating pulsar HerX-1 (refs 4, 20, 28). But it would require the slowly rotating GX1+4 to have a surface magnetic field as strong as  $10^{14}$  G, which is rather unlikely.

A better alternative is to assume a structural change in the accretion dynamics. During the rapid spin-up, accretion must have occurred by Roche-lobe overflow, leading to the formation of a stable accretion disk and hence efficient angular momentum transfer<sup>23</sup>. Considering that spin-down episodes are commonly seen among wind-fed X-ray pulsars<sup>1,3,4,24,25</sup>, it seems reasonable to assume that presently GX1+4 is powered by capture from a dense, slow, stellar wind, from the M6III companion, as originally suggested by Davidsen *et al.*<sup>13</sup>. Then the pulsar may sometimes receive a negative secular torque from the accreting

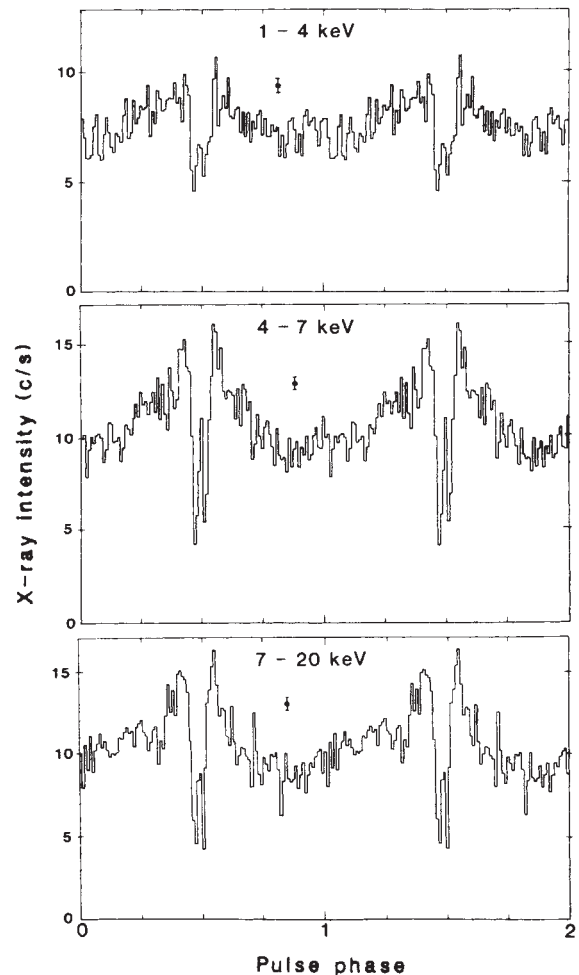


Fig. 3 Folded pulse profiles of GX1+4 in three energy bands, after background subtraction. For clarity, each profile is repeated for two cycles.

matter, through formation of an inversely rotating accretion disk<sup>26</sup>. A similar picture may also apply to transient X-ray pulsars<sup>4,27</sup> which experience large changes in  $\dot{M}$ . A more detailed examination of the problem, together with spectral studies, will be published elsewhere.

**Note added in proof:** A follow-up observation of GX1+4, made on 27 March 1988 with Ginga, gave  $P = 111.59 \pm 0.02$  s thus confirming the spin-down at an average rate of  $\dot{P} = 4.3 \times 10^{-8} \text{ s s}^{-1}$ .

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## The extended solar activity cycle

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The solar cycle has been defined in terms of a sequential periodic variation in sunspot numbers, the period being the interval between successive minima, currently averaging 11.2 years. But a number of observations have indicated that the activity cycle may begin at higher latitudes before the emergence of the first sunspots of the new cycle. Here we report results from sunspot cycle 21 concerning the ephemeral active regions, the coronal green-line emission and the torsional oscillation signal, which confirm the earlier suggestions. In particular, we report the appearance of a high-latitude population of ephemeral active regions in the declining phase of sunspot cycle 21, with orientations that tend to favour those for cycle 22 rather than 21. Taken together, these data indicate that sunspot activity is simply the main phase of a more extended cycle that begins at high latitudes before the maximum of a given sunspot cycle and progresses towards the equator during the next 18–22 yr, merging with the conventional 'butterfly diagram' (the plot of the latitudes of emerging sunspots against time) as it enters sunspot latitudes. We suggest that this extended cycle may be understood in the perspective of a model of giant convective rolls that generate dynamo waves propagating from pole to equator.

The small overlap of successive sunspot cycles in the butterfly diagram has long been known. But during the 1950s, coronal observers<sup>1–3</sup> noted that, in addition to the strong maxima in the emission of the 5,303-Å wavelength (green) line at sunspot latitudes, a zone of enhanced emission appears at high latitudes in each hemisphere several years before the beginning of the sunspot cycle and migrates equatorwards, reaching latitude ~40° in coincidence with the appearance of new-cycle spots at this latitude, apparently extending the butterfly diagram back in time to higher latitudes. Similar suggestions have been made by Leroy and Noens<sup>4</sup>, following a study of the variance of the 5,303-Å coronal emissivity from 1944 to 1974.

During sunspot cycle 20, Martin and Harvey<sup>5–7</sup> studied the properties of small magnetic bipoles without sunspots, ephemeral active regions (ERs), and found that they followed the declining number of active regions during the latter part of the cycle, although minimum occurred one or two years earlier for ERs. Further, they identified a high-latitude band of ERs in 1973 and 1975 and also noted a small but identifiable statistical tendency for the high-latitude ERs to follow the Hale–Nicholson orientation law for cycle 21 rather than 20. In a different context, Legrand and Simon<sup>8</sup> noted that enhanced geomagnetic activity related to the solar cycle arises from two components, one, the high-speed wind streams originating from high-latitude coronal

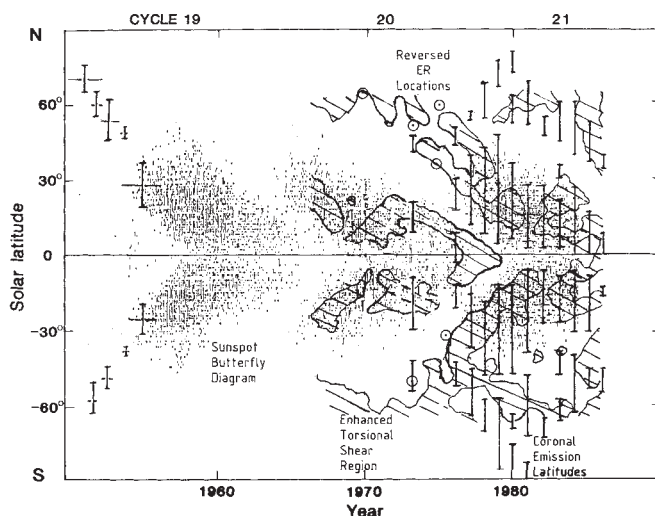


Fig. 1 Superimposed on the Mount Wilson butterfly diagrams for cycles 19, 20 and 21 are the greater-than-average shear zones of the torsional oscillations pattern<sup>11</sup> from 1967 to 1984, indicated by the hatched region within the contour lines, together with the green line emission maxima at high latitudes before cycle 19, from data given by Trellis<sup>2</sup>, and in 1973 and from 1976 to 1986, from Altrock's data. The vertical extent of the line represents the latitude range of significant emission during a given year. The circles represent the typical locations of predominantly reversed polarity ERs observed during cycle 20 by Martin and Harvey<sup>7</sup>.

holes and the other from sunspot activity. They pointed out that the high-latitude streams are closely related to coronal holes which form shortly after the polar field reversal and last for 9 or 10 yr, suggesting that these streams mark the beginning of a new cycle. In this regard, it has been suggested that the formation of coronal holes, shortly after closure of the polar crown gap, is related to the magnetic polarity reversal (P. S. McIntosh, personal communication).

Another important result was the discovery<sup>9,10</sup> of latitude bands of faster-than-average and slower-than-average rotation relative to the mean differential rotation for a given latitude, called the torsional oscillations. The faster bands first appear at polar latitudes before sunspot maximum and move progressively towards the equator over a period of 22 yr. They noted that, as the bands enter sunspot latitudes, the poleward shear boundary of the faster band corresponds to the locus of the centre-of-gravity of the newly forming active regions.

Thus several different groups, working in very different areas of solar activity, have independently suggested that their data indicate or are related to the presence of a high-latitude component of solar activity, in which the large-scale velocity patterns may also be involved.

We now discuss new results relating to sunspot cycles 21 and 22. The Mount Wilson velocity data have been further analysed<sup>11</sup> to derive the torsional shear (the derivative of the torsional pattern with respect to latitude). The latitude zones of greater-than-average shear between 1967 and 1986 are shown on the synoptic chart in Fig. 1 as hatched regions against a background of the Mount Wilson sunspot butterfly diagrams for cycles 19, 20 and 21. Three branches of this pattern may be found in either hemisphere. The first, a low-latitude branch from 1967 to 1979, closely matches the butterfly diagram for cycle 20. The second is first identified at high latitudes in 1967 and progresses continuously to lower latitudes, where it connects with the butterfly diagram for cycle 21 and accompanies it towards the equator. The third begins at high latitudes in 1979, although in the southern hemisphere it appears to be connected by a bifurcation to the second branch. It seems likely that this third branch will connect with the butterfly diagram for cycle 22, but this remains to be tested.



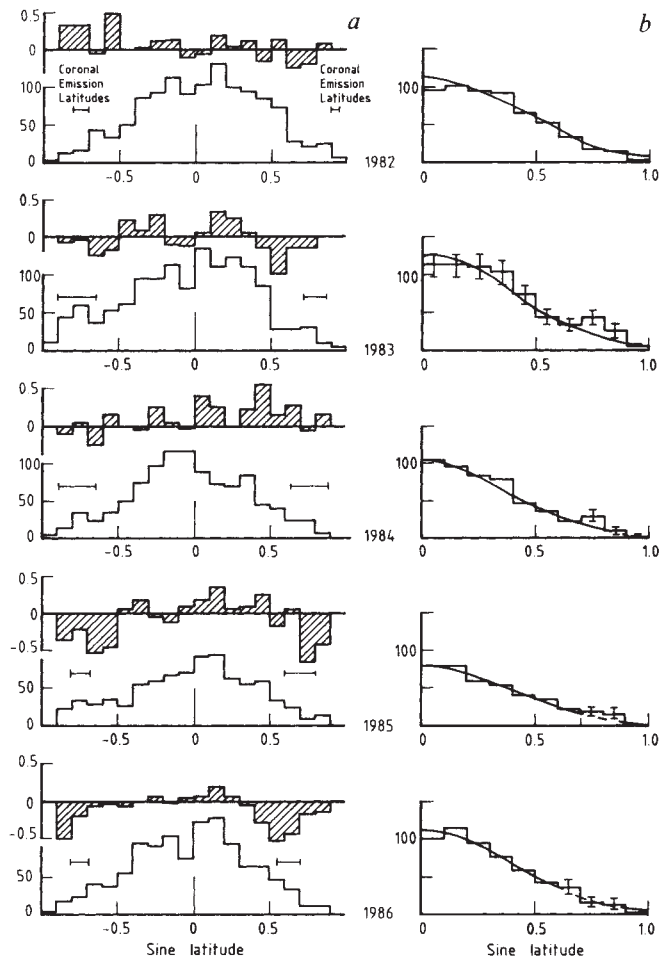
Again, we have obtained photoelectric maps of the corona in the 5,303 line, averaged over several rotations and extending over 13 yr. The latitude ranges of significant emission for 1973, and from 1976 to 1986, are also shown in Fig. 1, together with the corresponding emission data obtained by Trellis<sup>2</sup> before sunspot cycle 19. One component of the coronal emission clearly corresponds to the second branch of the torsional pattern, not only at sunspot latitudes but also at higher latitudes in 1973. However, from 1980 a high-latitude component of the emission is seen to follow the third branch of the torsional pattern, displaying an equatorward progressing pattern similar to that of Trellis<sup>2</sup>. This component appears to be linked to the second branch by a poleward-moving component between 1975 and 1980, which may also be related to a similar branch of the torsional pattern. These branches may reflect the poleward motion of the large-scale flux reported by Howard and LaBonte<sup>12</sup>.

Finally, we have analysed the latitude and orientation distributions of the ERs occurring during the declining phase of sunspot cycle 21, and histograms representing these distributions are shown in Fig. 2a for the years 1982–86. The unhatched histograms represent the total number of ERs, and the hatched histograms represent the ratio  $(P - R)/(P + R)$ , where  $P$  is the number of ERs with orientation appropriate to cycle 21 for each latitude box, and  $R$  is the number showing reverse orientation. Also shown for comparison are the latitude ranges of high-latitude coronal emission. The observed ER populations have not been corrected for a visibility function, and thus the lower-latitude populations are more heavily weighted. Nevertheless, subsidiary high-latitude maxima, or extensions of the ER populations, may be distinguished between 1982 and 1985, particularly in the southern hemisphere, at latitudes corresponding to the coronal emission. Further, as indicated by negative values  $(P - R)/(P + R)$  in 1983, 1985 and 1986, the high-latitude populations have a preference for orientations contrary to the Hale–Nicholson law for cycle 21, but consistent with that for cycle 22.

To investigate the significance of these high-latitude features, we have averaged the total ER populations for both hemispheres (for each latitude bin) and fitted a normal (or gaussian) distribution of the form  $N = N_0 \exp(-\sin^2 \lambda / \sigma^2)$  to the region  $\sin \lambda \leq 0.5$ , below which the effect of any high-latitude population would be negligible. Here  $\sigma$  is chosen so that the distributions correspond at the point of steepest descent, taken to be  $\sin \lambda = 0.5$  (0.45 in 1985 and 1986) and  $N_0$  by a least squares fit below 0.5. The gaussian distribution is then extrapolated over the rest of the range and is shown, together with the averaged observed distributions, in Fig. 2b.

The standard deviation of the actual populations from the fitted gaussians in the range  $\sin \lambda \leq 0.5$  is  $\sim 15\%$ , and provides an estimate for the random fluctuations in the populations. Below latitude  $30^\circ$ , where the populations are  $\sim 100$ , the error bars in Fig. 2b are drawn at  $\pm 15$ . But as the populations,  $N$ , decrease at higher latitudes, the percentage random fluctuations will be larger, increasing as  $N^{-1/2}$ , and the error bars are given by  $\pm 15 \times (N/100)^{1/2}$ . Populations in the range  $0.9 \leq \sin \lambda \leq 1.0$  are too small to estimate an error.

The tail of the gaussian provides a convenient example of a monotonically decreasing distribution, and it is clear that the departures of the high-latitude features of the observed distributions from the gaussians exceed the error bars by significant amounts in 1983, 1984, and to a lesser extent, in 1985. Indeed, in three cases, the departures are roughly twice the estimated standard error, which represents a 90% probability that the departures are real. This provides evidence for the existence of a distinct high-latitude ER population and is further supported by the preference for reversed, rather than normal, orientations at higher latitudes, which is evident in Fig. 2a. In 1982 there appears to be no significant high-latitude population, but Fig. 2a indicates that somewhat smaller subsidiary maxima occur in different latitude bins in opposite hemispheres and are thus



**Fig. 2** a, The unhatched histograms represent the total ER populations,  $P + R$ , plotted against sine (latitude) for the years 1982–1986. The hatched histograms show the relative excess of normally oriented ERs (with respect to cycle 21),  $(P - R)/(P + R)$ . Thus, negative values indicate a preference for orientations appropriate to cycle 22. Also shown are the high-latitude ranges of the coronal emission for these years. b, The histograms show the populations for the sine-latitude ranges of Fig. 2a averaged over north and south hemispheres. The error bars, determined as described in the text, are shown for all latitude bins in 1983 and at selected latitudes in other years. The curve represents the corresponding gaussian distributions.

concealed by the averaging process in Fig. 2b.

The ER results for cycle 20 are therefore qualitatively reproduced. Further, although the high-latitude ER populations do not exhibit an unambiguous latitude drift, the relationship between these populations and the coronal emission and the torsional shear pattern, both of which exhibit clear and continuous drifts towards the equator between 1980 and 1986, supports the hypothesis that these phenomena are the forerunners of sunspot cycle 22 and are, in fact, part of an extended activity cycle (of which sunspot activity is the major component) which begins at high latitudes near sunspot maximum of the previous cycle and progresses towards the equator over a period of 18–22 yr. In addition, this extended cycle is quite different from the 22 yr magnetic cycle, which simply consists of two 11-yr sunspot cycles.

This new concept is of considerable importance for our understanding of the cycle. Some models, such as that of Babcock<sup>13</sup>, require the establishment of a new global poloidal field at the conclusion of each 11-yr cycle to generate the toroidal magnetic fields for the next 11-yr cycle. But if the next extended cycle is

already beginning at the poles while its predecessor is approaching the maximum of its sunspot phase, such that they are overlapped for more than half of their ~22-yr lifetimes, then these models are no longer tenable. Dynamo wave models, such as those proposed by Parker<sup>14,15</sup>, in which two sub-surface toroidal bands of magnetic field coexist within each hemisphere, would seem to be more appropriate, but, using current estimates of the sign of the radial acceleration and of cyclonic convection, Parker<sup>16</sup> has shown that these dynamo waves propagate polewards, a result he has characterized as the 'dynamo dilemma'.

As the torsional oscillation signal links the sunspot and the high-latitude phases of activity together, the origin of this signal may hold the key to an understanding of the extended cycle. Howard and LaBonte<sup>9,10</sup> regarded it as a periodic torsional motion about the solar rotation axis, and, although they could not identify the perturbing force, they suggested that the restoring force might be a strong sub-surface magnetic field. Yoshimura<sup>17</sup> and Schussler<sup>18</sup> independently suggested that it arose from the magnetic Lorentz force as a back reaction to the generation of active regions, although this does not explain the velocity pattern at high-latitudes.

Elsewhere we have proposed<sup>19</sup> that the torsional oscillations represent the surface signature of a system of giant sub-surface azimuthal or doughnut-shaped convection rolls migrating from pole to equator. Although studies<sup>20,21</sup> of the onset of convection in a compressible rotating fluid indicate that the largest eddies should take the form of 'banana-shaped' cells elongated in the meridional (that is, north-south) direction, an investigation by Eltayeb<sup>22</sup>, independently confirmed by D. J. Galloway and C. A. Jones (personal communication), shows that in the presence of a toroidal magnetic field the mode of convection is determined by the Elsasser number  $\Lambda = B^2/2\mu\eta\Omega$ , where  $B$  is the intensity of the toroidal field,  $\mu$  the permeability,  $\rho$  the plasma density,  $\eta$  the magnetic diffusivity and  $\Omega$  the angular velocity. For small values of  $\Lambda$ , banana cells are favoured, but for values in excess of a critical value (~1), the onset of convection should take the form of azimuthal rolls. Although there are difficulties in the accurate determination of  $\Lambda$  for the Sun, Galloway and Jones argue that it should be comparable with the critical value. Further, they show that the critical value of  $\Lambda$  is likely to be less near the poles than at the equator between 1980 and 1986, which is consistent with the picture of complete azimuthal rolls near the poles breaking up into giant cells at lower latitudes<sup>19</sup>.

In a recent commentary, Howard<sup>23</sup> has discussed the possible alternatives, concluding that the picture is still confused. But elsewhere<sup>24</sup> we have shown that, in the deep convection zone, the doughnut roll model can maintain an equatorward-propagating dynamo wave, generating two sub-surface magnetic toroids in each hemisphere. These propagate from high to low latitudes on a timescale appropriate to the cycle, and, by associating the high-latitude toroid with the high-latitude ER population and related phenomena, we have a mechanism which may account for the extended activity cycle. Further, we suggest that the thermal shadow created above the toroid (as proposed by Parker<sup>25</sup>) provides a suitable location for the downflow of a convective roll system so that, as the dynamo wave drives the magnetic toroid towards the equator, it is accompanied by the moving pattern of convective rolls, at least during its high-latitude phase. In this regard, it is of interest that Kuhn *et al.*<sup>26</sup> have suggested that the limb-temperature minima, which they found near latitude 50° in 1983, 1984 and 1985, may be associated with the large-scale convection.

We conclude that the evidence from cycle 21 supports and extends the earlier suggestions of an extended activity cycle, and, to place this in some perspective, we propose a system of azimuthal convective rolls forming near the poles every eleven years. This system provides the driving mechanism for a dynamo wave and generates the toroidal magnetic fields. At high-latitudes it is assumed that the rolls are complete and the magnetic toroid gives rise to only the weaker forms of activity (the ERs

and the coronal emission features). As the wave progresses to lower latitudes, we suggest that the rolls break up into giant cells having an east-west velocity component, and the large active regions are generated from the sub-surface toroid, possibly after the manner suggested by McIntosh and Wilson<sup>27</sup>. Thus a coherent picture of a plausible mode of operation of the extended cycle and the large-scale convection is beginning to emerge and should provide a basis for further testing during the current sunspot cycle.

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## A measurement of the altitude variation of greenhouse radiation from CFC-12

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Here we report measurements that provide new experimental evidence to support climate model predictions of the greenhouse effect. We demonstrate that emission of chlorofluorocarbons (CFC) into the atmosphere by anthropogenic usage has modified the long-wave radiation budget of the atmosphere by ~0.1%. The 10.8- $\mu\text{m}$  band of CFC-12 is situated in the thermal window of the atmosphere and hence has a powerful greenhouse effect. Measurements of the downward long-wave infrared flux in this band have been made with a filter spectrometer, covering the spectral range 9-14  $\mu\text{m}$ , as a function of height over an altitude range 5-25 km. The balloon ascent took place on 19 May 1985 from Palestine, Texas at 31° N under clear sky conditions. The observed radiance profile is consistent with a model flux calculation using the observed temperature profile. The downward flux measured at the tropopause was 0.008  $\text{W m}^{-2}$ ; this will be potentially valuable for comparison with unpublished climate model fluxes for mid-latitudes in summer. The measured downward greenhouse flux at 5 km was 0.05  $\text{W m}^{-2}$ , which is consistent with published climate model values for the radiation change at the surface, after accounting for the pressure difference.



There have been many climate model studies of the predicted greenhouse effect and several attempts by data analysis to detect the change in surface temperature, predicted to be  $\sim 0.5$  degrees by 1985 (ref. 1). This paper reports a measurement of a demonstrable change in the long-wave flux budget due to the increase in atmospheric concentrations of CFC-12. As the changes in the infrared radiation budget will precede the actual surface temperature warming by about 50 years due to the thermal lag of the oceans<sup>2,3</sup>, the monitoring of the flux changes could potentially improve early warning of the greenhouse effect. The direct detection of a radiative flux change is recognized to be important by Kiehl<sup>4</sup>. That is, the detection of such a flux change provides additional experimental evidence that the theoretical predictions of climate models could take place. What is still uncertain is whether or not these radiative flux changes will significantly affect global-mean temperatures, and climate in general. Will the forcing signal be negated by, for example, negative feedbacks such as those associated with cloud amount, type and optical property changes? The feedback response of the climate system will also be easier to quantify with this new data on the radiative forcing. This type of tropospheric measurement of downward long-wave flux will also be useful to correlate with planned satellite measurements of outgoing long-wave radiation<sup>5</sup>.

In the greenhouse effect, incident short-wave energy from the Sun warms the Earth during the sunlit hours. This heating is balanced by outgoing long-wave infrared blackbody radiation 24 hours a day. The atmosphere emits downward infrared radiation towards the Earth's surface; this downward flux warms the Earth and is known as the greenhouse radiation because the Earth's surface temperature tends to increase in response to this flux. In this paper a direct measurement of the greenhouse-radiation flux term due to CFC-12 in the  $10.8\text{-}\mu\text{m}$  band is reported. The observed flux represents a well-defined increment to the radiation budget as CFC-12 does not occur naturally in the atmosphere.

The flux changes were measured as a function of altitude from a balloon flight in summer at mid-latitude. The flux measurements were taken with a cryogenically cooled, circular variable filter spectrometer<sup>6</sup>. The instrument measures infrared radiance from 5 to 8 micrometres in the lower wavelength range and radiance from 8 to 14 micrometres in the upper wavelength range. It has a noise equivalent sensitivity of  $5 \times 10^{-8} \text{ W cm}^{-2} \mu\text{m}^{-1} \text{ sr}^{-1}$ .

The infrared spectra were measured at 0.5 km intervals during the ascent phase of the balloon flight. The data reported here were measured on from 0300 to 0500 hours on 19 May 1985 at Palestine, Texas at  $31^\circ \text{N}$ . The temperature profile was measured on a series of four radiosonde flights to altitudes over 40 km and spread over a period of two days, including the flight time.

An increment to the greenhouse radiation is most effective in the 10 to  $12\text{-}\mu\text{m}$  window. The spectrum observed from 6 km is shown in Fig. 1. The long-wave window is bounded by the  $9.8\text{-}\mu\text{m}$  ozone band on the lower side, and by the 13 to  $16\text{-}\mu\text{m}$  band of carbon dioxide on the long-wavelength side. The band of nitric acid is present at  $11.3\text{-}\mu\text{m}$ . CFC-11 has a feature at  $11.8\text{-}\mu\text{m}$  and CFC-12 has a feature at  $10.8\text{-}\mu\text{m}$ . There is a continuum absorption from 9 to  $14\text{-}\mu\text{m}$  due to water vapour. If the observed spectrum could be simulated with a radiation code, then the contributions of the naturally occurring gases such as ozone, carbon dioxide and water vapour could be removed to leave the contributions from CFC-11 and CFC-12. The radiation code chosen to do this was Lowtran 6 (ref. 7). We have some confidence in the Lowtran 6 code which has been extensively verified by comparison with measured atmospheric spectra<sup>7</sup>; in addition, we have found that Lowtran 6 generally provides an excellent simulation of our measured spectra as it has about the same spectral resolution of 15 wavenumbers.

The observed spectrum has been simulated with the Lowtran 6 code<sup>7</sup> and the comparison is shown in Fig. 1. When the simulated spectrum is subtracted from the observed spectrum

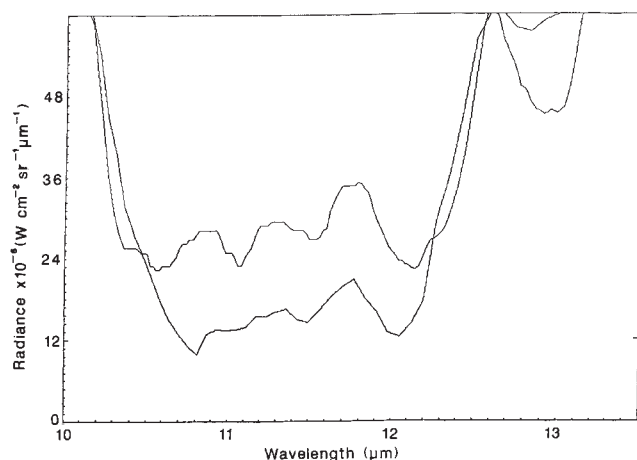


Fig. 1 The observed downward long wave radiance spectrum at 6 km on 19 May 1985. A simulated spectrum calculated with Lowtran at 6 km is shown for comparison.

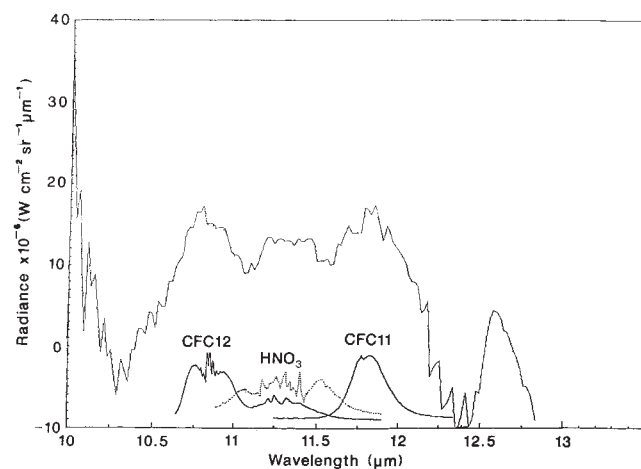


Fig. 2 The measured radiance spectrum at 6 km after the removal of ozone, carbon dioxide, water vapour and nitric acid by subtraction of the Lowtran simulation. The laboratory spectra of CFC-12, CFC-11 and  $\text{HNO}_3$  are shown at the bottom for interpretive purposes.

at 6 km, ozone, carbon dioxide, water vapour and even nitric acid should be removed; the subtracted spectrum is shown in Fig. 2. The features of CFC-11 at  $11.8\text{-}\mu\text{m}$  and CFC-12 at  $10.8\text{-}\mu\text{m}$  are now obvious when compared with the laboratory spectra for these gases at the bottom of Fig. 2. There appears to be some residual continuum due to water vapour left in the spectrum. The amplitude of the CFC-12 feature was determined from this spectrum. The amplitudes of the CFC-12 feature at all measured altitudes were determined and the radiances plotted as a function of altitude. In Fig. 3, the flux in the CFC-12  $10.8\text{-}\mu\text{m}$  band has been plotted as a function of altitude from 5 to 25 km. A conversion from the measured radiance units (microwatts per  $\text{cm}^2\text{-steradian-micrometre}$ ) to flux (watts per  $\text{m}^2$ ) has been performed by integrating over wavelength of the feature ( $10.5\text{--}10.9\text{-}\mu\text{m}$ ) and by performing an integration over solid angle. The angle integration was approximated by considering the zenith viewing angle of the radiometer ( $70^\circ$ ) and the field of view of the spectral radiometer ( $15^\circ$ ). (This conversion factor turned out to be 2.3.) Azimuthal symmetry in the radiation field was also assumed.

The simple Atmospheric Environment Service<sup>1</sup> radiation code was used to simulate the radiance which should have been

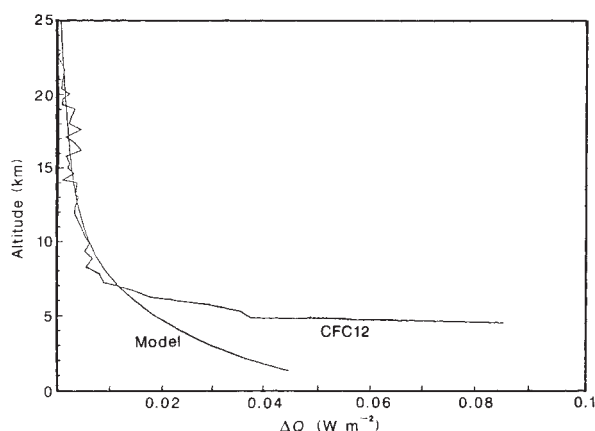


Fig. 3 The variation of measured downward flux in the 10.8- $\mu\text{m}$  band of CFC-12 as a function of altitude, as determined from the measured spectra over the range of altitudes from 5 to 25 km.

observed for the measured temperature profile and a mixing ratio of 400 p.p.t.v. (parts per trillion by volume) of CFC-12; this comparison is shown in Fig. 3. The observed radiance is consistent with the radiance model at all altitudes above 6 km. Below this altitude, there appears to be additional contributions to the radiance profile above the expected radiation from CFC-12. This may be due to the fact that it is difficult to compensate accurately for water vapour at lower altitudes. Less likely, these contributions could be due to hitherto unsuspected greenhouse gases or errors in the radiation code. In any case, these results demonstrate the potential importance of real measurements of the greenhouse radiation; these could improve on the present situation, where complete reliance is placed on the predictions of unverified climate models that large climate perturbations will occur in the future.

The observed flux changes at various levels are among the parameters calculated in some climate models of the greenhouse effect. In particular, the flux values at the tropopause and at the surface are considered to be important values. Ramanathan *et al.*<sup>8</sup> and Dickinson and Cicerone<sup>1</sup> predict values of net flux changes from CFC-12 at the tropopause of  $0.12 \text{ W m}^{-2}$ ; this should not be compared directly with the measured value of the downward flux at the tropopause from Fig. 3 of  $0.008 \text{ watts m}^{-2}$ . The surface flux change predicted by the model of Kiehl<sup>4</sup> is  $0.12 \text{ W m}^{-2}$ ; this is not inconsistent with the observed value of  $0.05 \text{ W m}^{-2}$  at 5 km as the flux should double from 5 km to the surface due to the increase in atmospheric pressure (that is, 0.10 versus  $0.12 \text{ W m}^{-2}$ ).

Below this altitude of 5 km, the contributions from other gases such as water vapour are difficult to compensate for properly with Lowtran, and the measured flux increases rapidly until the measured flux at 2 km is over  $0.2 \text{ watts m}^{-2}$ . The amount of water vapour present is crucial at the lower levels near the surface, as the correction becomes large under summer conditions. The measurements were conducted under clear sky conditions, but it is quite humid in Texas in May. One also needs to be cautious about the intercomparison as the model is a one-dimensional calculation at a fictional latitude. A series of inter-comparisons of many measurements at various seasons and latitudes would be required to conduct a comprehensive test of the theory. In particular, measurements under cold, dry winter conditions would ameliorate the water vapour problem. But the magnitude of the observed signal is consistent with theory and represents a preliminary experimental confirmation of the predicted greenhouse radiation change. As the radiation changes are predicted to lead the temperature changes by about 50 years<sup>9</sup>, these types of measurement could potentially provide an early warning of potential future temperature increases.

A detection and quantitative measurements of the change in greenhouse radiation due to CFC-12 have been conducted. This provides a graphic demonstration that the anthropogenic emission of greenhouse gases is changing the atmosphere. The observed fluxes are consistent with the tropopause and surface values predicted by climate models for 1985 (ref. 4). This detection of the greenhouse radiation change provides credence to the model predictions that CFCs will produce a serious greenhouse effect in the future. It also demonstrates that measurement and even monitoring of radiation balance changes is feasible.

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## Cosmogenic $^{32}\text{P}$ and $^{33}\text{P}$ used as tracers to study phosphorus recycling in the upper ocean

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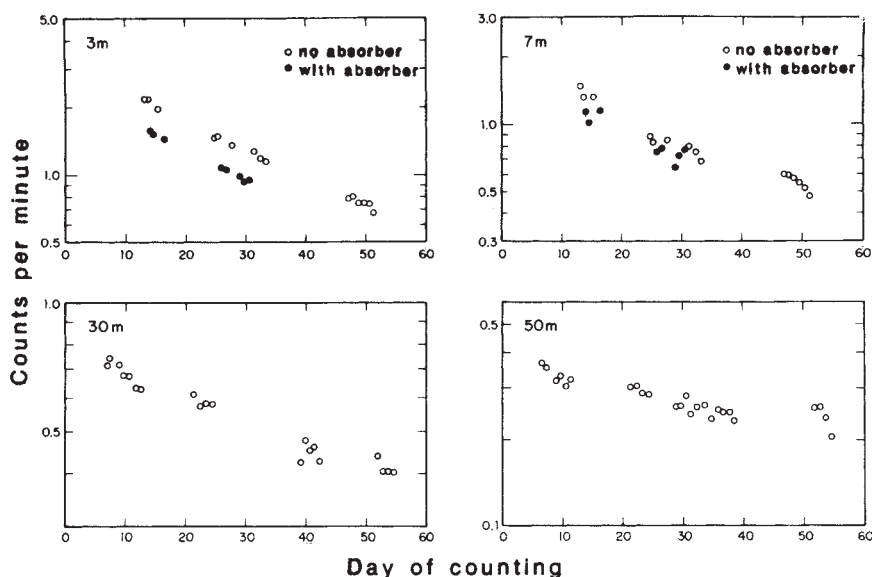
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The elements C, N and P are mostly biologically recycled in the upper ocean. Consequently, nutrient cycling and food web dynamics are central problems in marine biology and chemistry. The natural stable isotopes  $^{13}\text{C}$  and  $^{15}\text{N}$  and the radioisotopes  $^3\text{H}$ ,  $^{14}\text{C}$  and  $^{32}\text{P}$  have been used to estimate rates of *in situ* photosynthesis, nucleic acid synthesis<sup>1,2</sup> and new production<sup>3–8</sup>, but previously there was no practical way of studying the gross character of nutrient cycles in their natural environment. We have now developed a method based on two naturally occurring radioisotopes,  $^{32}\text{P}$  (half-life, 14.3 days) and  $^{33}\text{P}$  (half-life, 25.3 days) to study phosphorus recycling in the upper ocean. We have studied their concentrations in the dissolved inorganic phosphorus, dissolved organic phosphorus and particulate organic phosphorus from waters within and below the mixed layer. These isotopes permit study of P cycling on the timescales compatible with those involved in biogeochemical processes and in trophic interactions within the food web.

Cosmic rays produce the two radionuclides  $^{32}\text{P}$  and  $^{33}\text{P}$  in the atmosphere by nuclear reactions with argon nuclei<sup>9</sup>, as well as directly in the oceans by reactions with Cl, S and K nuclei<sup>10</sup>. Their concentrations in wet precipitation were studied<sup>11</sup> in the 1950s. Their tropospheric production is injected into the oceans by wet precipitations. The amount produced directly *in situ* in the mixed layer of the oceans is estimated<sup>10</sup> to be less than 20% of that added by wet precipitations; the oceanic production is confined to the top 5 m depth. The integrated average inventories of  $^{32}\text{P}$  and  $^{33}\text{P}$  in the ocean column from these sources are estimated to be 190 and 380 atoms per  $\text{cm}^2$  respectively ( $^{32}\text{P}$  is also produced *in situ* by decay of cosmogenic  $^{32}\text{Si}$ , but this

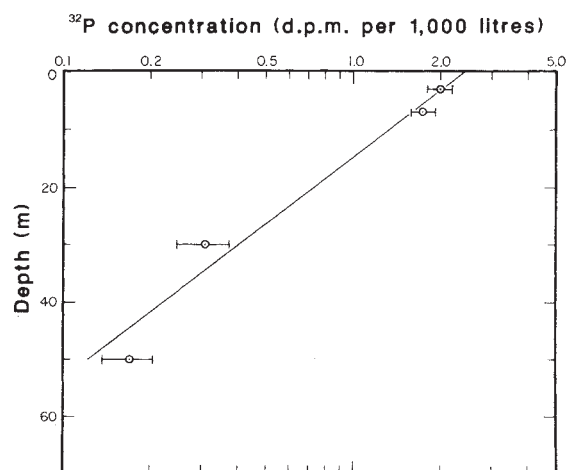


**Fig. 1** Observed gross beta-counting rates of radiochemical phosphorus extracts from four DIP samples (SCBS 205), as a function of time. The countings with absorber (filled circles) were made to estimate  $^{33}\text{P}$  activities. The counter backgrounds were in the range 0.25–0.35 c.p.m. Results of  $^{32}\text{P}$  and  $^{33}\text{P}$  concentrations are given in Table 1.



source is not important<sup>10</sup> at depths <500 m). This low concentration has probably prevented investigation of their usefulness as natural tracers. Indeed, the task is enormous as it involves extraction of dissolved inorganic and organic phosphorus (DIP and DOP) and particulate organic phosphorus (POP) from large volumes of sea water, and counting the low activities of these nuclides. But considering the usefulness of studying natural tracers over artificial tracers, and the fact that two isotopes of phosphorus with suitable half-lives occur naturally, it seemed worthwhile to make the necessary technical advances. Our technique also adapts to routine analyses. We measured DIP, DOP and POP in waters off Santa Catalina Island (latitude 33°17.3' N, longitude 118°10.4' W) at the Southern California Bight Study (SCBS) Station 205; the station lies on the CALCOFI line 90.

The DIP was extracted on board by passing water successively through a 5- $\mu\text{m}$  particulate filter cartridge and  $\text{Fe}(\text{OH})_3$ -coated acrilan fibres<sup>10</sup> of 20- $\mu\text{m}$  diameter from depths of 3, 7, 30 and 50 m. The coated fibres have been successfully used earlier for *in situ* extraction of dissolved inorganic silicon<sup>12</sup> from sea water; we found<sup>10</sup> it more convenient and effective for extraction of DIP than other techniques. Some DOP may have been picked by the coated fibres and this aspect is under investigation. Estimate of the DIP concentration was based on the method of Strickland and Parsons<sup>13</sup> and may be a slight overestimate because the method also measures some labile organophosphorus compounds<sup>14</sup>. Dissolved organic phosphorus, primarily nucleotides, was removed by passing surface sea water through two activated charcoal columns (0.3 l) after trapping particulate matter on a 5  $\mu\text{m}$  filter cartridge. The chemical procedures for the extraction of radiochemically pure phosphorus and the measurements of  $^{32}\text{P}$  and  $^{33}\text{P}$  activities using a Q-gas flow counter have been described<sup>10,11</sup>. The plankton samples were collected



**Fig. 2** The measured  $^{32}\text{P}$  concentrations in the DIP profile samples collected from SCBS 205 (September 1987) are plotted as a function of depth. The best-fit exponential line is drawn through the points.

using a 1-m-diameter, 505- $\mu\text{m}$  net. The net was towed obliquely from the surface to ~100 m; wire release was 200 m.

Results of measurements of  $^{32}\text{P}$  and  $^{33}\text{P}$  activities in DIP are presented in Table 1, and plotted in Fig. 1 for  $^{32}\text{P}$ . Here our emphasis was on the determination of  $^{32}\text{P}$  activity;  $^{33}\text{P}$  activity can be determined more accurately with larger counters<sup>10</sup>. Hydrographic data for our station are available from CALCOFI studies<sup>15</sup>, and SCBS expeditions since 1974<sup>16</sup>. The depth of the mixed layer during the sampling period was 30–35 m, showing an annual range in depth of between 25–40 m. The measured  $^{32}\text{P}$  activity in surface waters, 2.0 d.p.m.  $\text{m}^{-3}$ , is about 2–3 times that observed in four surface samples collected off San Diego. The  $^{32}\text{P}$  activity is seen to decrease exponentially with depth with the half-concentration depth,  $Z_{1/2}$  of 11.5 m (Fig. 2). The total amount of  $^{32}\text{P}$  in DIP in a vertical column, estimated on the basis of exponential fit, is determined to be  $4.1 \times 10^{-3}$  d.p.m.  $\text{cm}^{-2}$ , or 120  $^{32}\text{P}$  atoms per  $\text{cm}^2$ . (The corresponding inventory of  $^{32}\text{P}$  in the POP is estimated to be <3% of this amount.) This value is 64% of the inventory estimated from production.

The observed exponential decrease of  $^{32}\text{P}$  and  $^{33}\text{P}$  shows that the mixed layer is not well mixed in the timescale of 2–3 weeks at our station. The concentration gradient is caused by two

**Table 1** Measured  $^{32}\text{P}$  and  $^{33}\text{P}$  activities in DIP at SCBS 205, CALCOFI line 90 on 26–28 September 1987

| Water depth (m) | DIP concentration ( $\mu\text{g atom l}^{-1}$ ) | Effective volume of water processed ( $\text{m}^3$ ) | Net observed activity at first counting (c.p.m.) | Activity (d.p.m. $\text{m}^{-3}$ ) |                 |
|-----------------|-------------------------------------------------|------------------------------------------------------|--------------------------------------------------|------------------------------------|-----------------|
|                 |                                                 |                                                      |                                                  | $^{32}\text{P}$                    | $^{33}\text{P}$ |
| 3               | 0.30                                            | 4.2                                                  | 1.75                                             | $2.00 \pm 0.20$                    | $2.52 \pm 1.0$  |
| 7               | 0.32                                            | 3.3                                                  | 1.04                                             | $1.75 \pm 0.20$                    | $1.36 \pm 0.5$  |
| 30              | 0.31                                            | 4.6                                                  | 0.39                                             | $0.31 \pm 0.06$                    | $0.40 \pm 0.2$  |
| 50              | 0.46                                            | 2.9                                                  | 0.15                                             | $0.17 \pm 0.03$                    | $0.25 \pm 0.1$  |

factors: radioactive decay during downward mixing and upward mixing of  $^{32}\text{P}$ -poor waters through the thermocline.

The  $^{33}\text{P}/^{32}\text{P}$  activity ratios in the DIP (Table 1) are higher than at production, 0.99, considering both the atmospheric flux and *in situ* production. The ~1.3 times increased ratio in the mixed layer corresponds to a mean residence time of 20 days for DIP in the mixed layer, not inconsistent with the observed deficiency in the  $^{32}\text{P}$  inventory.

The results for zooplankton show large variations for the September collections (Table 2). During night collections, the

**Table 2**  $^{32}\text{P}$  activities in zooplankton examples collected at SCBS 205

| Sample code | Collection Date | Time  | Observed activity* (c.p.m.) | $^{32}\text{P}$ d.p.m. g $\text{P}^{-1}$ |
|-------------|-----------------|-------|-----------------------------|------------------------------------------|
| ZN1.SC1     | 21 July 1987    | 22-03 | 0.11                        | $8.0 \pm 1.6$                            |
| ZN2.SC1     | 21 July 1987    | 22-03 | 0.11                        | $8.9 \pm 1.6$                            |
| MXN.SC2     | 27 Sept. 1987   | 03-06 | 0.37                        | $45.1 \pm 5.0$                           |
| ZON.SC2     | 27 Sept. 1987   | 03-06 | 0.58                        | $39.1 \pm 4.0$                           |
| MD1.SC2     | 27 Sept. 1987   | 07-09 | 0.07                        | $11.0 \pm 2.0$                           |
| MD2.SC2     | 27 Sept. 1987   | 07-09 | 0.31                        | $100 \pm 10$                             |

ZN1.SC1 and ZN2.SC1 samples are primarily euphausiids and calanoid copepods; the remaining samples were mostly calanoid copepods. Samples with codes starting with Z are primarily zooplankton; others are mixed with some phytoplankton.

\* Net observed activity at first counting.

catch was higher as a result of zooplankton migrating up from depths. (One purpose of the investigations was to see if  $^{32}\text{P}$  and  $^{33}\text{P}$  activities differed significantly in the day and night catches.) The night samples collected during 21 July have a very low  $^{32}\text{P}$ , <10 d.p.m.  $^{32}\text{P}$  per g P. These values translate to a value of 0.08 d.p.m.  $^{32}\text{P}$  per  $\text{m}^3$  sea water, taking the measured DIP concentrations for the mixed layer. This conversion is useful as it allows comparison of  $^{32}\text{P}$  activity with that found in surface waters where primary production occurs and with regions of different phosphorus concentrations. The highest value observed (MD2.SC2; Table 2) yields a value of 0.96 d.p.m. per  $\text{m}^3$  sea water. Four zooplankton samples, one from the Celtic Sea and three from the Bedford basin, gave values of 0.3, 0.35, 0.30 and 1.00 d.p.m. per  $\text{m}^3$  sea water respectively. These values are of the same order as those found in our collections on 27 September. The observed variability would probably also be expected. The much lower activity for the collection of 21 July probably reflects the dominating contributions from euphausiids that feed on deeper 'older' food.

Samples of phytoplankton from the Bedford basin<sup>10</sup> show  $^{32}\text{P}$  specific activities ~3 times higher than zooplankton collected on the same day. The results are in accord with a relatively short turnover time of phosphorus in the phytoplankton (<2 weeks) and a highly variable and much larger turnover time for the zooplankton samples (>30-40 days).

Two samples of DOP were collected using the activated charcoal method<sup>17</sup>. The charcoal was eluted with 1:1 HCl before use. The results for 3 m and 12 m depth are  $\geq 0.01$  and  $\geq 0.26$  d.p.m.  $^{32}\text{P}$   $\text{m}^{-3}$  sea water; these values are lower limits because of uncertainties in the intrinsic phosphorus content of the activated charcoal. (We can rule out the possibility of any appreciable contamination from POP on the basis of the total amount of POP trapped in the particulate filter used. The filter was very efficient, the particulates being confined to within 5 mm of the outer layer of the filter cartridge.) The significant  $^{32}\text{P}$  concentration of the 12 m DOP sample indicates a fairly rapid exchange in the upper ocean, apparently a result of the activity of the microbial food web<sup>18</sup>.

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## Interannual variability in climate and fisheries in Tasmania

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Whereas the interannual variability of the climate and fisheries of the Northern Hemisphere has been extensively documented<sup>1</sup>, very little is known about the interannual variability in the fisheries of the Southern Pacific Region. Recent work in the Northern Hemisphere has demonstrated the close relations between interannual variability in climate, the timing of events in the water column, the structure of food chains and recruitment to both marine and freshwater fisheries<sup>2,3</sup>. Forty years (1945-85) of observations at a coastal station (Maria Island, 42°36' S, 148°16' E) in Tasmania showed strong interannual variability in sea-surface temperatures. Maria Island is close to the region of convergence of the surface currents, on the equatorial side of the Subtropical Convergence (STC) water mass boundary<sup>4</sup>. The spring bloom was often extended by as much as three months in some years. Previous work<sup>4</sup> has not offered any explanation for the observed interannual variability and does not show any links with commercial fisheries. Here we explain the reason for the interannual climatic and oceanic variability in Tasmania and show the links between climate and the fisheries.

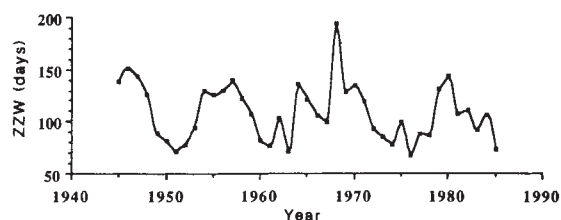
The westerly wind belt of the Southern Hemisphere has a dominant influence on the climate of Tasmania. The position of the main westerly wind belt over Tasmania is determined by



the position of the continental high-pressure area over Australia and hence by El Niño–Southern Oscillation (ENSO) events<sup>5</sup>. There was a clear cyclical pattern in these zonal westerly winds (ZWW) with a mean periodicity of 11 yr (Fig. 1, Table 2). Interannual changes in ZWW were correlated with annual mean atmospheric pressures at Hobart and Macquarie Island (Table 1). Throughout the 40-yr record, the annual mean atmospheric pressure in Hobart was low the year before peaks in pressure at Darwin<sup>6</sup>; the year before ENSO events. Decreases in annual mean pressure at Hobart and Macquarie Island were associated with increases in ZWW over Tasmania, increases in rainfall from the zonal westerlies, increases in the level of Great Lake (in the Central Highlands of Tasmania) and reductions in the annual maximum summer air temperatures near the lake at Shannon (Table 1, Fig. 2). The zonal westerlies bring gales and cold, wet weather to Tasmania. The maximum summer air temperature at Shannon has, however, risen by 2 °C during the period 1945–85 without a long-term decrease in westerlies (Figs 1 and 2).

Changes in the level of Great Lake (elevation 1,030 m) were significantly correlated with ZWW and rainfall at Shannon over the period 1945–1985 (Table 1, Fig. 2). The lake-level data indicated that the cyclical pattern of fluctuation in the incidence of ZWW has been consistent since at least 1916 when records began. Changes in rainfall and lake level controlled variations in both the numbers and mean weight of trout caught in the lake. The census data obtained from returns of anglers' questionnaires showed a negative correlation between total angling effort (and therefore total harvest of trout) and ZWW at Great Lake ( $n = 13$ ,  $r = -0.71$ ). Angling effort was lower during cold, windy, wet weather (high ZWW), which resulted in changes in angling mortality from year to year. Mean weight of trout in the anglers' catch was negatively correlated with the minimum lake level of the same year. When lake levels decreased, larger fish resident on the deep water *Chara* beds were more frequently caught from shore and by shallow trolling (P.D. and R. Sloane, in preparation).

The numbers of fish of first spawning age (3 yr) at Liawenee Canal, Great Lake, were significantly correlated with mean maximum air temperature at Shannon over the period 1945–85 (Table 1). A high correlation was found between the lake tem-



**Fig. 1** The interannual variability in the total number of days of zonal westerly winds over Tasmania. Westerly wind data for Tasmania was obtained by classification of the 09:00 surface synoptic pressure chart for the southern Australian region. Daily charts from 1945 to 1985 were classified into eight types: (1) anticyclone over the Bight; (2) anticyclone over the Tasman Sea; (3) anticyclone over Tasmania; (4) zonal westerlies; (5) cyclone to the north of Tasmania; (6) cyclone to the east of Tasmania; (7) cyclone to the west of Tasmania; (8) cyclone over Tasmania. The classification assumed that each weather system dominated the entire Island for the day. Thus it was possible to derive a time series of yearly totals of each weather type. In classifying a weather type as being 'zonal westerlies' the following criteria were used. (1) The presence of a marked N–S pressure gradient, with isobars mostly aligned E–W. Cases with an orientation of NW–SE and SW–NE were also included. (2) The presence of more than one cold front embedded in the westerly stream. Cold fronts follow one another in rapid succession in the westerly streams over Tasmania. (3) Total duration of the weather system of more than one day. Typical duration is a few days.

peratures and maximum air temperature at Shannon ( $r = 0.76$ ,  $n = 61$ ). The gradual rise in temperature at Shannon, and hence the rise in lake temperatures over the period 1945–85, led to a rise in the proportion of 3-yr-old trout in the spawning populations. This indicates that the interannual variability of ZWW, the ambient temperature and the age structure of the trout in spawning migrations at Great Lake were directly related (Fig. 2). The relation was particularly strong for female brown trout.

Strong westerly winds also drive colder, nutrient-rich subantarctic waters up the east coast of Tasmania in summer<sup>4</sup>. Increased westerly winds disrupt the pycnocline throughout the summer months, replenishing the nitrate in surface waters by

**Table 1** Statistically significant correlations between climatological, limnological, oceanographic and fisheries data assuming no time lags

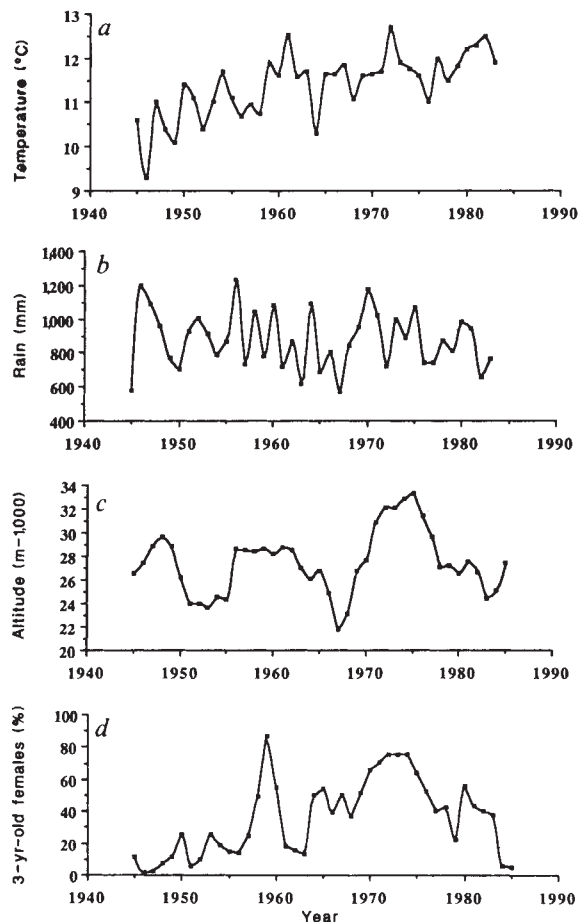
|     | DAR | HOB   | MAC   | ZWW    | MAX   | RAI  | GLL  | DEL   | TRO   | SPR   | MAR    | TAS    | NSW    | NZ   | TUN    | SEA   |
|-----|-----|-------|-------|--------|-------|------|------|-------|-------|-------|--------|--------|--------|------|--------|-------|
| DAR | —   | 0.41† | -0.69 |        |       |      |      |       |       |       |        | 0.32‡  |        |      |        |       |
| HOB |     | —     |       | -0.65† | 0.73† |      |      | -0.33 |       |       |        | 0.72†  | -0.57† |      |        |       |
| MAC |     |       | —     | -0.67  |       |      | 0.47 |       |       | -0.40 | 0.43   | -0.36‡ |        |      | -0.47  | -0.33 |
| ZWW |     |       |       | —      | -0.28 | 0.30 |      | 0.43  |       | 0.50  |        | -0.35† | 0.62†  | 0.30 |        |       |
| MAX |     |       |       |        | —     |      |      |       | 0.53* |       |        | 0.66†  |        |      |        |       |
| RAI |     |       |       |        |       | —    |      | 0.46  |       |       |        |        |        |      |        |       |
| GLL |     |       |       |        |       |      | —    | 0.31  | 0.40  | -0.50 | 0.31   |        |        |      |        |       |
| DEL |     |       |       |        |       |      |      | —     |       |       |        |        |        |      |        |       |
| TRO |     |       |       |        |       |      |      |       | —     |       | 0.40   | 0.59*  |        |      |        |       |
| SPR |     |       |       |        |       |      |      |       |       | —     | -0.70† |        |        |      |        |       |
| MAR |     |       |       |        |       |      |      |       |       |       | —      |        |        | 0.41 | -0.43† | 0.37  |
| TAS |     |       |       |        |       |      |      |       |       |       |        | —      | -0.32† |      |        |       |
| NSW |     |       |       |        |       |      |      |       |       |       |        |        | —      |      |        |       |
| NZ  |     |       |       |        |       |      |      |       |       |       |        |        |        | —    |        |       |
| TUN |     |       |       |        |       |      |      |       |       |       |        |        |        |      | —      |       |
| SEA |     |       |       |        |       |      |      |       |       |       |        |        |        |      |        | —     |

\* Data showing long-term trend.

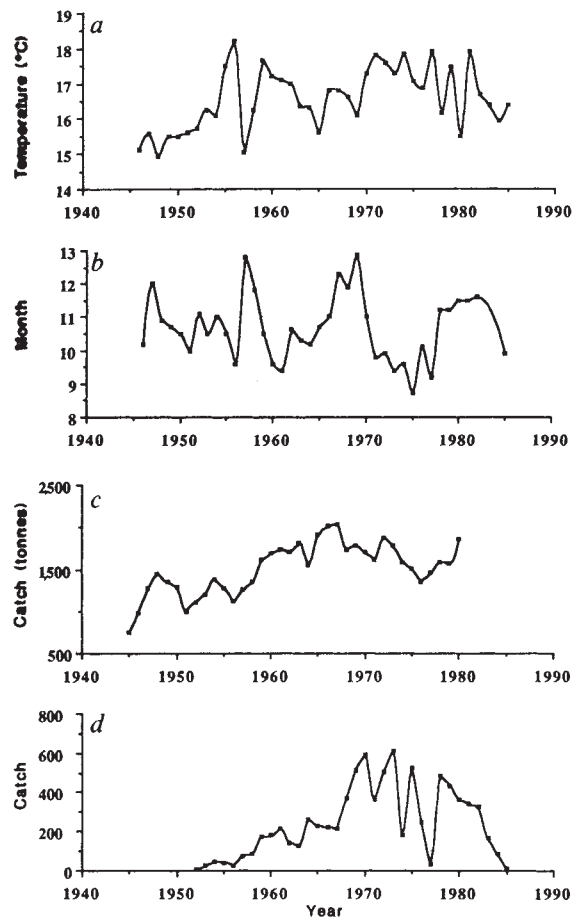
† Data analysed from 1950 to 1980.

‡ Two lowest MAC years deleted as outliers from trends DAR, HOB, MAC.

Statistical analyses were performed by GENSTAT and SYSTAT. Series were rendered stationary by differencing. Methods as in refs 20 and 21. DAR, HOB, MAC, atmospheric pressure at Darwin, Hobart and Macquarie Island. ZWW, annual total of zonal westerly winds over Tasmania. MAX, maximum temperature at Shannon. RAI, annual rainfall at Shannon. GLL, Great Lake level. DEL, change in Great Lake level. TRO, percentage of 3-yr-old female brown trout. SPR, timing of spring bloom on Maria Island. MAR, maximum summer sea-surface temperature at Maria Island. TAS, NSW, NZ, total lobster catch (weight), Tasmania, New South Wales, New Zealand (Otago). TUN, New South Wales tuna catch (weight). SEA, abundance of leopard seals Macquarie Island.



**Fig. 2** Climatological and limnological data for the Tasmanian Central Highlands, 1945–85. Time series of *a*, mean maximum annual air temperatures at Shannon weather station; *b*, annual rainfall at Shannon; *c*, mean annual level of Great Lake as altitude above sea level (data from records of the Bureau of Meteorology, Hobart and the Hydro-Electric Commission, Hobart); *d*, percentage of 3-yr-old female brown trout in the spawning migrations at Liawenee Canal, Great Lake. All fish were trapped in a fixed diversion trap and aged by scale reading<sup>19</sup>.



**Fig. 3** Time series of oceanographic and fisheries data from Tasmania and New South Wales. *a*, Maximum summer sea surface temperatures at Maria Island, Tasmania; *b*, timing of the spring bloom at Maria Island as determined by methods in ref. 4 (8, August; 12, December; 13, January); *c*, total Tasmanian spiny lobster catch (tonnes); *d*, New South Wales southern bluefin tuna catch (total numbers). Oceanographic data was obtained from CSIRO records and was collected by methods detailed in ref. 4. Fisheries data were obtained from records kept by Australian Commonwealth agencies and State Department of Fisheries.

vertical mixing. The annual totals of zonal westerly winds were correlated with the timing and duration of the spring bloom in Tasmanian coastal waters at Maria Island and with reductions in maximum summer water temperatures (Fig. 3, Table 1). The seasonal cycle of phytoplankton was therefore controlled in precisely the way described by Sverdrup<sup>7</sup>, with frequent windy periods leading to frequent restarting of the spring bloom and sustained productivity throughout the summer.

The local wind field determines the position of the convergence in the surface currents on the northern edge of the water mass boundary between Australia and New Zealand as Wyrski suggested<sup>8</sup>. In ENSO years the surface convergence appears to move north so that the southern Tasman Sea is cooler by 3 °C across 3° of latitude<sup>9</sup>. Crosscorrelations between climatological and oceanographic parameters and fisheries catches in Tasmania, New South Wales and New Zealand (Tables 1 and 2) revealed a widespread effect that was correlated with changes in the Darwin–Macquarie Island pressure gradient and the incidence of ZWW over Tasmania. Both the Tasmanian and New Zealand populations of spiny (rock) lobsters are caught on the southern side of the surface convergence and show positive correlations with increased temperatures at Maria Island (Tables 1 and 2). The Tasmanian and New Zealand

(Otago) populations of the lobsters (*Jasus novaehollandiae* and *J. edwardsii*) are now thought to be conspecific<sup>10</sup> and are caught at ~7 and 3 years old, respectively. The significant crosscorrelations between the catches of lobsters on both sides of the Tasman Sea and the biologically realistic time lags indicate a widespread effect of climate and ocean circulation and the possibility of long-range larval transport. A different species of *Jasus* (*J. verreauxi*) is caught on the northern side of the STC in New South Wales and on the northern tip of the North Island of New Zealand<sup>13</sup> and shows a correlation of opposite sign to those species on the southern side of the STC convergence. *J. verreauxi* also occurs on the tip of the north island of New Zealand; also north of the STC<sup>11</sup>.

Two effects are evident in the fisheries data: an immediate effect of effort and a lagged effect of recruitment. Fishing effort increased in periods of high atmospheric pressure and calm seas. This explains the high correlations (Table 1) between the Tasmanian spiny lobster (*Jasus novaehollandiae*) catch and air temperatures and pressures in Tasmania in the same year. The significant time lags of 3–7 yr (Table 2) between oceanographic events and the lobster catch data are consistent with the known life histories of the organisms and the mean ages of animals caught.



As the West Wind Drift is driven by the zonal westerlies, it is not surprising to find a correlation between ZWW and the recruitment of stocks of lobsters over a wide area. Circumpolar larval transport in the '*lalandii*' group of *Jasus* species is a possibility<sup>12</sup>. The phyllosomata larvae spend  $\leq 2$  yr at sea, so circumpolar transport in the West Wind Drift is possibly via the tips of the continents and seamounts on the northern edge of the Southern Ocean. It has been suggested that recruitment of *J. tristani* at Vema Seamount results from transport from Tristan da Cunha<sup>13</sup>. Pearce and Phillips<sup>14</sup> showed that ENSO events and the recruitment of the western rock lobster in Western Australia were correlated.

Other stocks in Tasmanian waters also show large interannual variability. Harrison's<sup>15</sup> data on scallop catches in Tasmania from the 1940s to the 1960s indicate the possibility of similar climatological control by ZWW. For scallops, years of high incidence of ZWW appear to favour good recruitment perhaps by a link between high productivity in high ZWW years, and increase in spawning and high larval survivorship. Data on the New South Wales catch of southern bluefin tuna (*Thunnus maccoyii*) from 1950 to 1984 indicates that sea-surface temperature affects the distribution of the fish (Fig. 3, Table 1)<sup>16</sup>. Southern bluefin tuna migrate across the southern coast of Australia as far north as the 20 °C isotherm. In years of low pressure at Macquarie Island (increased ZWW) and low sea surface temperature at Maria Island bring more fish into the area of the fishery. Leopard seals (*Hydrurga leptonyx*) are residents of the Antarctic pack ice but juveniles disperse northwards in winter as far as subtropical waters<sup>17</sup>. The abundance of leopard seals recorded at Macquarie Island<sup>17</sup> from 1949 to 1979 is also a function of atmospheric pressure on the island (Table 1) as well as sea surface temperatures at Maria Island in Tasmania. This also indicates an interaction between ENSO, ZWW, the strength of the West Wind Drift in the Southern Ocean and the migration of some species of marine mammals in the sub-antarctic.

The air and sea surface temperature data both show consistent rises in temperature in Tasmania during the period 1945–85 (Figs 2 and 3). This was noted a decade ago in southeast Australia and the same effect has been noted in Auckland, New Zealand<sup>16,18</sup>. It appears that southeastern Australia is located so as to be very sensitive to changes in the atmospheric and oceanic circulation associated with ENSO events<sup>6</sup>. Increased pressure at Darwin was correlated with late spring blooms in Tasmania which therefore tended to be more pronounced in ENSO years (Figs 1 and 3). These changes, together with longer-

term changes in the air and sea temperatures in the southern Tasman Sea area, have had, and are likely to continue to have, significant effects on the fisheries in this area.

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## Mid-Holocene rainfall in the Negev Desert from $^{13}\text{C}$ of land snail shell organic matter

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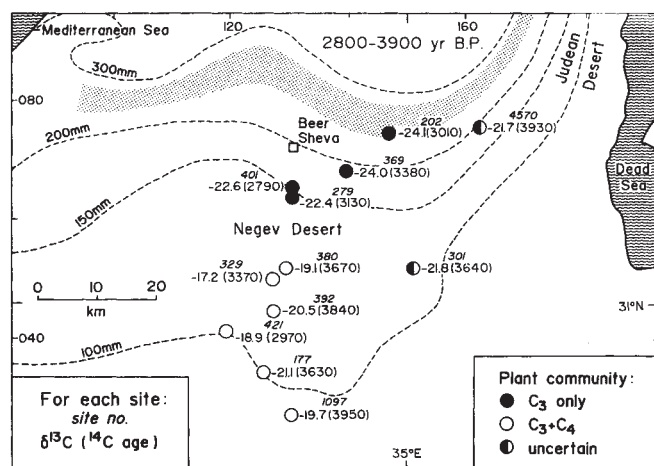
Analysis of the  $^{13}\text{C}/^{12}\text{C}$  ratio of organic matter in fossil bones has been used in a number of studies for reconstruction of palaeodiets, especially the presence or absence of plants having a  $\text{C}_4$  photosynthetic pathway (enriched in  $^{13}\text{C}$  relative to  $\text{C}_3$  plants)<sup>1,2</sup>. This isotope method has also been used to detect organic matter derived from  $\text{C}_4$  plants in materials such as sediments<sup>3–5</sup> and desert rock varnishes<sup>6</sup>. In the present study, the  $^{13}\text{C}/^{12}\text{C}$  ratio of the organic matrix of fossil land snail shells from the Northern Negev Desert in southern Israel was used to map out the distribution of  $\text{C}_4$  shrubs in the past. Because in this region  $\text{C}_4$  shrubs are generally restricted to the arid zone ( $\leq 280$  mm mean annual rainfall), shifts in rainfall patterns could be inferred from the changes in plant distributions. Analysis of land snail shells dated to the period ~2,800–4,000 yr BP showed that during this time the northern limit of  $\text{C}_4$  shrubs was shifted 20–30 km south of its present position, implying substantially wetter conditions in the Northern Negev than occur there at present.

The Northern Negev is a region of strong rainfall gradients, representing the transition zone from the hyperarid Saharan-Arabian desert belt in the south to the more humid Mediterranean climate of the north (Fig. 1). Within this desert boundary region (100–400 mm mean annual rainfall), a survey was carried out to document the distribution of  $\text{C}_4$  shrubs and forbs (herbs that are not grasses). Results (Table 1) indicate that in areas receiving  $\geq 290$  mm mean annual rainfall, nearly all (94%) of the plant communities consist entirely of  $\text{C}_3$  plants. In areas receiving  $\leq 230$  mm, most (90%) of the plant communities contain  $\text{C}_4$  species (nearly all are shrubs in the Chenopodiaceae), whereas within the narrow transition zone of 240–280 mm, a mixture of pure  $\text{C}_3$  and mixed  $\text{C}_3 + \text{C}_4$  plant communities occurs. The few pure  $\text{C}_3$  communities that occur at  $\leq 230$  mm are generally dominated by *Zygophyllum dumosum* Boiss. or *Artemisia herba-alba* Asso, and are found in the more southerly area (100–125 mm) on hill slopes where the soil cover is thin<sup>7</sup>. This relation of the distribution of  $\text{C}_4$  Chenopodiaceae to moisture conditions has also been documented on a worldwide scale<sup>8</sup>.

**Table 2** Statistically significant crosscorrelations between climatological, oceanographic and fisheries data

| Autocorrelations                                                |          |          |          |
|-----------------------------------------------------------------|----------|----------|----------|
|                                                                 | <i>r</i> | lag (yr) | <i>n</i> |
| Number of days ZWW                                              | 0.40     | 11       | 29       |
| Crosscorrelations                                               |          |          |          |
| Hobart atmospheric pressure against Darwin atmospheric pressure | −0.46    | 1        | 40       |
| Annual rainfall at Shannon against Great Lake level             | 0.37     | 1        | 40       |
| Maximum temperature (°C) Maria Island against:                  |          |          |          |
| total weight, Tasmanian Lobsters                                | 0.37     | 7        | 30       |
| total weight, New Zealand lobsters                              | 0.38     | 3        | 30       |
| total weight NSW lobsters                                       | −0.35    | 5        | 30       |
| Total weight, Tasmanian lobsters against                        |          |          |          |
| total weight, New Zealand lobsters                              | 0.49     | 4        | 30       |

Time series-methods are as described in refs 20 and 21. Series were rendered stationary by differencing.



**Fig. 1** Map of the Northern Negev in southern Israel, giving location, site number,  $\delta^{13}\text{C}$  of shell organic matter, and  $^{14}\text{C}$  age of fossil samples of the land snail *Trochoidea seetzeni*.  $\delta^{13}\text{C}$  data are interpreted as representing pure  $\text{C}_3$  plant communities, mixed  $\text{C}_3+\text{C}_4$  plant communities, or an uncertain plant community, based on the calibration in Table 2. The present-day transition zone between the predominantly  $\text{C}_3$  plant communities (to the north) and the predominantly mixed  $\text{C}_3+\text{C}_4$  plant communities (to the south) is shown by stippling. Present-day mean annual rainfall isohyets are shown. Latitude, longitude, and local grid coordinates are given.

Because of this close relationship of the distribution of  $\text{C}_4$  chenopodes to rainfall, it is possible to reconstruct past rainfall patterns on the basis of the past distribution of  $\text{C}_4$  chenopodes. In this study I have used isotope analysis of the organic matrix of shells of the land snail *Trochoidea seetzeni*, a species with an extensive Holocene fossil record and which at present lives in abundance in the Negev. *T. seetzeni* feeds on senescent or freshly dead plant material, its diet serving as the source of compounds used in synthesis of the organic matrix of its shell. In terrestrial molluscs the organic matrix is composed of proteins<sup>9</sup> with some polysaccharides<sup>10</sup>.

To assess the possibility of predicting the presence or absence of  $\text{C}_4$  plants on the basis of the  $^{13}\text{C}$  content of shell organics,  $^{13}\text{C}$  was analysed in shell organics extracted from live specimens collected from pure  $\text{C}_3$  and mixed  $\text{C}_3+\text{C}_4$  plant communities. Included were samples from sites containing the predominant  $\text{C}_4$  chenopode species of the region. The results (Table 2) show that in every case a distinct difference in  $\delta^{13}\text{C}$  was found between snails from pure  $\text{C}_3$  plant communities and those from mixed  $\text{C}_3+\text{C}_4$  communities. The former all show  $\delta^{13}\text{C}$  values  $\leq -22.7\%$ , whereas the latter show  $\delta^{13}\text{C}$  values more positive than  $-22.7\%$ . This is the pattern expected as a result of the enriched  $\delta^{13}\text{C}$  values characteristic of  $\text{C}_4$  plants. The results indicate that wherever  $\text{C}_4$  plants (shrubs and forbs) are available, they are eaten by the snails and consequently reflected in the  $\delta^{13}\text{C}$  values of the snail shell organics. The most enriched value ( $-15.1\%$ ) was from snails from a plant community consisting almost entirely of  $\text{C}_4$  plants. Although there is a tendency for more enriched values to occur in snail samples from communities having higher  $\text{C}_4$  plant abundance, a direct correspondence is not found. This may be because of monthly or annual variations in biomass of annuals (almost all  $\text{C}_3$  species) or because of preferential feeding by the snails on certain species.

To apply this calibration of  $\delta^{13}\text{C}$  values to fossil snails, an additional factor must be taken into account: the depletion of  $^{13}\text{C}$  of atmospheric  $\text{CO}_2$  (and consequently in plants) due to the burning of fossil fuels since the nineteenth century. Based on tree ring  $\delta^{13}\text{C}$  values, this decrease in  $^{13}\text{C}$  amounts to  $1.0\%$  (ref. 11). Thus for pre-nineteenth-century snails, the expected  $\delta^{13}\text{C}$  cutoff value between pure  $\text{C}_3$  and mixed  $\text{C}_3+\text{C}_4$  plant communities is  $-21.7\%$ . As a result of analytical uncertainty

and the uncertainty of the correction for the industrial effect, an overall uncertainty of  $\pm 0.5\%$  for the cutoff value is used for interpretation of the fossils. Thus values of  $\delta^{13}\text{C} < -22.2\%$  are taken to indicate pure  $\text{C}_3$  plant (shrubs + forb) communities, whereas values of  $\delta^{13}\text{C} > -21.2\%$  are taken to indicate the presence of  $\text{C}_4$  plants. Samples having intermediate values ( $-22.2$  to  $-21.2\%$ ) are disregarded, as they provide no definite information on the plant communities.

Fossil *Trochoidea seetzeni* were collected from a variety of types of deposits in the Northern Negev: middens accumulated in rodent burrows (eight of the samples reported here), fluvial deposits (one), colluvial deposits (two), and from sediments in archaeological sites (one) (details given in ref. 12). Before analysis, the age uniformity of each sample was evaluated by the uniformity of the ratio of the amino acid epimers D-alloisoleucine/L-isoleucine (A/I) among six specimens from each deposit<sup>12</sup>. This methodology is based on the strong dependence of variation in A/I ratios on age in Negev land snails<sup>13</sup>. Samples were dated by  $^{14}\text{C}$  analysis of shell carbonate (11–25 g) after thorough cleaning to remove secondary carbonate<sup>12</sup>. Ages were calculated from  $^{14}\text{C}$  activity corrected for isotope fractionation<sup>14</sup> and for the shell carbonate age anomaly that results from incorporation of ancient carbon derived from digestion of carbonates in sediments<sup>14</sup>. A figure of 1,500 yr was used for this latter correction<sup>12</sup>, but the variability of this age anomaly ( $\pm 230$  yr) introduces an age uncertainty in addition to the uncertainty of the laboratory measurement of radiocarbon<sup>14</sup>. The overall error for the samples reported here varies from  $\pm 250$  to 310 yr.

Here I report the results for twelve samples of snail shells whose radiocarbon ages fall within the period 4,000–2,800 yr BP. Of the twelve samples, ten gave organic  $^{13}\text{C}$  results that allowed definite identification of the plant community in which the snails lived. It is seen (Fig. 1) that only pure  $\text{C}_3$  plant communities were found in the region that now receives 150 to 250 mm mean annual rainfall—an area that now supports plant communities mostly containing  $\text{C}_4$  species (Table 1). On the other hand, communities having  $\text{C}_4$  plants were present at all sites in the region that now receives 100 to 130 mm mean annual rainfall. The results indicate that during this period the transition zone from plant communities containing  $\text{C}_4$  species to nearly all pure  $\text{C}_3$  communities lay between what are now the 150 and 120 mm rainfall isohyets. By analogy with the occurrence of the present day transition zone between the 240–280 mm isohyets, the rainfall isohyets during the period ~3,000–4,000 yr BP must have been located in a position 20–30 km south of their present positions. This implies roughly twofold increase in mean annual rainfall for this part of the Northern Negev relative to the present. The geographical consistency of the distribution of

**Table 1** Survey of the distribution of plant communities with only  $\text{C}_3$  plants and with both  $\text{C}_3+\text{C}_4$  plants, in relation to mean annual rainfall

| Mean annual rainfall* (mm) | Number of sites with $\text{C}_3$ only | Number of sites with $\text{C}_3+\text{C}_4$ | Total number of sites | % Sites with $\text{C}_3$ only ( $\pm \text{s.e.}$ )† |
|----------------------------|----------------------------------------|----------------------------------------------|-----------------------|-------------------------------------------------------|
| 330–400                    | 29                                     | 2                                            | 31                    | 94 $\pm$ 3%                                           |
| 290–320                    | 20                                     | 1                                            | 21                    |                                                       |
| 240–280                    | 11                                     | 12                                           | 23                    | 48 $\pm$ 10%                                          |
| 200–230                    | 0                                      | 18                                           | 18                    | 10 $\pm$ 3%                                           |
| 100–190                    | 10                                     | 70                                           | 80                    |                                                       |

Only sites not showing signs of burning or heavy grazing were selected. Plants were classified as  $\text{C}_3$  or  $\text{C}_4$  according to published data<sup>28–30</sup> and  $^{13}\text{C}$  analysis of possible  $\text{C}_4$  taxa for which no published data are available. Grasses were not included in the survey. The survey area included the Northern Negev and the Judean Desert (its continuation to the north-east).

\* Based on ref. 31.

† Standard error of estimated per cent based on binomial distribution.



plant communities during this period points to the stability of plant distributions, and hence a long-term stability of rainfall. Short-term fluctuations (on the decade scale) cannot be detected by this method, because plant distributions are not likely to respond to climatic change on so short a timescale. These wet conditions persisted at least until  $2,800 \pm 250$  yr BP (the data of sample 401, which indicates the presence of a pure  $C_3$  community at the present 150 mm isohyet). The northward shift of  $C_4$  plants since this time cannot be explained as a result of grazing, as this would tend to favour  $C_3$  plants: the shrub *Thymelaea hirsuta* (L.) Endl. and the lily *Asphodelus microcarpus* Salzm. and Viv., both  $C_3$  plants, are among the very few plant species not eaten by goats and sheep and generally predominate in overgrazed areas<sup>7</sup>. Grazing mainly causes changes in predominance of species and reduction of stature, rather than extirpation of plant species in a community<sup>7</sup>.

Palaeoclimatic evidence that is unambiguous and can definitely be placed within the time range considered here is rather scarce for the southern Levant region in general and is nonexistent for the Northern Negev. The frequency distribution of amino-acid epimer ratios of land snail shells in Holocene fluvial terraces in the Northern Negev indicates a peak of alluviation around mid-Holocene (about 6,000–4,000 yr BP), with a gradual decrease thereafter<sup>12</sup>. Although this could be taken to indicate decreasing rainfall, it may relate rather to decreased frequency of flooding, with little change in mean annual rainfall. Evidence of Holocene Dead Sea levels provides information on rainfall variations in the Dead Sea–Jordan Valley drainage basin, mainly to the north of the Dead Sea. From a high level of –280 m around 7,000–6,500 yr BP<sup>15</sup>, the Dead Sea had fallen to below –340 m by 5,000 yr BP (earliest age of tombs at Bab edh-Dhra<sup>16</sup>), and was below –396 m by the tenth century BC<sup>17</sup> (equivalent to a  $^{14}\text{C}$  age of 2,800 yr BP<sup>18</sup>), near the modern level of the Dead Sea (at about –400 m). Analysis of Dead Sea cores

indicates the deposition of considerable amounts of halite after  $4,410 \pm 320$  yr BP<sup>19</sup>. Both lines of evidence point to relatively dry conditions in the north during the period of about 4,000–3,000 yr BP considered here. Analysis of pollen from cores from the Hula Basin in northern Israel give conflicting results for this period: the pollen curve of Horowitz<sup>20</sup> shows a peak of relatively high arboreal pollen (mostly *Quercus*) during this period, whereas that of Tsukada<sup>21</sup> shows variable but generally low arboreal pollen (also mostly *Quercus*) for this period. The Dead Sea data can be reconciled with those presented here for the Northern Negev if the climatic changes resulted from a southward shifting of the rainfall isohyet patterns, rather than an overall increase of rainfall throughout the southern Levant. This shift would have had little effect on rainfall in the north, where north–south rainfall gradients are weak, but would have had a pronounced effect in the Northern Negev, where the rainfall gradient is very strong. This pattern could come about by a southward displacement or a weakening of the high pressure cell centred over the Arabian Desert, which tends to deflect storm systems toward the east, away from the Negev.

The period 4,000–2,800 yr BP (equivalent to calendric ages of 2500 to 1000 BC<sup>18</sup>) was one which saw the progressive decline of human civilization in the Northern Negev area. Just before this period, at the end of the Early Bronze II Period at about 2600 BC, Arad, the great city of the region, was abandoned<sup>22</sup>. At the end of the Early Bronze III Period at about 2300 BC<sup>15</sup>, the major tells along the northern border of the Negev were abandoned<sup>23</sup>. Following the end of Early Bronze IV (equivalent to Middle Bronze I), at about 2000 BC<sup>15</sup>, the Negev was abandoned altogether<sup>24</sup>, until the reappearance of settlements at the beginning of the Iron Age at around 1200 BC. The first two phases of depopulation have both been attributed to the advent of drier climatic conditions<sup>22,25,26</sup>. But the evidence presented here indicates that this region experienced relatively wet conditions during this period. It is possible that short-term droughts that did not produce changes in plant distributions could have been responsible for the events at the end of Early Bronze II and III, but the long period of absence of humans from the Negev following Early Bronze IV clearly cannot be attributed to dry conditions persisting over this span of time. Other explanations of a cultural nature must be sought.

One of the major advantages of the  $\delta^{13}\text{C}$  of land snail shell organics in palaeoclimatic studies is that they reflect strictly local environmental conditions and thus permit reconstruction of palaeoclimate on a fine geographic scale. Such fine-scale reconstructions have also been made on the basis of packrat middens (see ref. 27, for example). The use of strictly local palaeoclimatic indicators is particularly important in areas in which strong climatic gradients exist. Other commonly used palaeoclimatic methods integrate environmental conditions over too broad an area for meaningful reconstructions in such regions—for example, pollen analysis (pollen may be blown in from some distance away) and lake levels (which integrate conditions over the whole of the drainage basin). Land snails appear to be particularly good material for organic isotope studies because, although they contain only a small amount of organic carbon ( $\sim 0.03\%$  by weight), they are not prone to contamination by soil organics. This is because, unlike most bone material, they have a solid, non-porous structure. The  $^{13}\text{C}$  of soil organic matter has also been studied to identify the presence or absence of  $C_4$  plants in the past<sup>3–5</sup>, but this approach has the disadvantage that soil organics may be contaminated by penetration of roots during a later period. Unlike bone, the shells of land snails retain their organic matter well: no difference in the average organic content between early Holocene and late Holocene shells from the Negev has been found (G.A.G., unpublished data). Lack of diagenesis is also indicated by the regular increase of the ratio of the epimers D-alloisoleucine/L-isoleucine with age<sup>13</sup>. If differential loss of certain organic components occurred, this could lead to isotopic fractionation, so that

**Table 2**  $\delta^{13}\text{C}$  values of organic matter from shells of live-collected *Trochoidea seetzeni* from pure  $C_3$  and from mixed  $C_3 + C_4$  plant communities

| C <sub>3</sub> + C <sub>4</sub> plant communities |                           |                           | Pure C <sub>3</sub> plant communities |                           |
|---------------------------------------------------|---------------------------|---------------------------|---------------------------------------|---------------------------|
| Site coordinates                                  | C <sub>4</sub> species*   | $\delta^{13}\text{C}$ (‰) | Site coordinates                      | $\delta^{13}\text{C}$ (‰) |
| 190, 134                                          | <i>S.a.</i>               | –15.1                     | 155, 064                              | –22.7                     |
| 189, 139                                          | <i>H.a.</i> , <i>S.v.</i> | –17.1                     | 161, 079                              | –22.8                     |
| 139, 068                                          | <i>A.a.</i>               | –18.4                     | 183, 135                              | –22.9                     |
| 139, 041                                          | <i>H.s.</i>               | –19.5                     | 131, 092                              | –23.3                     |
| 131, 063                                          | <i>N.m.</i>               | –19.6                     | 183, 135                              | –23.3                     |
| 136, 037                                          | <i>H.s.</i>               | –19.9                     | 181, 135                              | –23.4                     |
| 144, 051                                          | <i>A.a.</i> , <i>H.s.</i> | –20.5                     | 112, 080                              | –24.8                     |
| 132, 051                                          | <i>N.m.</i> , <i>H.s.</i> | –21.5                     |                                       |                           |
| 131, 063                                          | <i>N.m.</i>               | –21.5                     |                                       |                           |
| 145, 078                                          | <i>N.m.</i> , <i>A.a.</i> | –22.0                     |                                       |                           |
| 129, 061                                          | <i>N.m.</i>               | –22.6                     |                                       |                           |

Cutoff value: –22.7‰.

Cutoff value (corrected for industrial effect)<sup>†</sup>: –21.7‰.

Each sample consists of 15–25 shells (10–15 g) collected from areas of c.  $10 \times 10$  m<sup>2</sup>. Site locations are given in local grid coordinates (E–W, N–S). The organics were prepared by dissolving the cleaned shells (10–15 g) in 6N HCl, centrifuging (10 min at 2,500 r.p.m.), washing the organic pellet with distilled water and centrifuging (repeated three times) and freeze-drying the pellet. CO<sub>2</sub> was prepared from the organics by heating them in the presence of CuO<sub>2</sub> in evacuated and sealed quartz tubes at 810 °C for 2 h. After purification of gas samples in a vacuum line,  $^{13}\text{C}/^{12}\text{C}$  was measured on a Varian M250 mass spectrometer.  $^{13}\text{C}$  values are expressed as  $\delta^{13}\text{C}$ , which is the difference (in per mil) between the sample and the PDB standard. Analytical uncertainty is  $\pm 0.15\%$ .

\* Plant species abbreviations are as follows: *A.a.*: *Anabasis articulata* (Forssk.) Moq.; *H.a.*: *Halogeton alopecuroides* (Del.) Moq.; *H.s.*: *Hamada scoparia* (Pomel) Iljin; *N.m.*: *Noaea mucronata* (Forssk.) Oschers. & Schweinf.; *S.a.*: *Suaeda asphaltica* (Boiss.) Boiss.; *S.v.*: *Salsola vermiculata* Forssk. ex J. F. Gmel.

<sup>†</sup> See text for explanation.

calibrations based on analysis of modern material would not be precisely applicable to fossil material.

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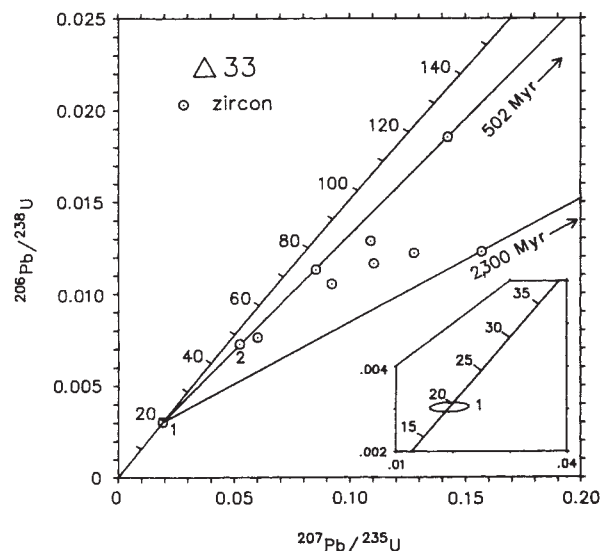


Fig. 1 Concordia diagram for ten zircon fractions from sample  $\Delta 33$ : data are bounded by two discordia from 19.5 Myr on concordia to upper intercepts of  $\sim 500$  and 2,300 Myr. Inset shows blowup of 2 sigma error ellipse for analysis of a single zircon crystal concordant at 19.5 Myr. Numerals 1 and 2 refer to zircon analyses in Table 1.

plots on concordia at  $19.5 \pm 0.4$  Myr. Eight monazite fractions lie on a line from an upper intercept of  $471 \pm 10$  Myr to the youngest fraction, which plots slightly above concordia at 21 Myr. The presence of excess  $^{206}\text{Pb}$  in the youngest monazite fractions indicates that the monazite data represent a mixing line between young magmatic monazite and an older inherited component, rather than a resetting of 471 Myr monazites at  $\sim 20$  Myr. These data suggest a closure temperature for Pb in monazite significantly higher than previously published estimates.

We have obtained separates of zircon and monazite from a weakly foliated, unmetamorphosed, two-mica tourmaline granite (sample  $\Delta 33$ ) which intrudes rocks that have been deformed in response to the Tertiary collision of India and Asia. This 30 kg sample was collected by B. C. Burchfiel, K. V. Hodges and L. Royden from north-east Everest ( $86^\circ 50' \text{ E}$ ,  $28^\circ 10' \text{ N}$ ) as part of their ongoing research on the tectonics of the Himalayas and southern Tibet. U-Pb analyses were obtained on carefully selected zircon and monazite fractions, using current procedures at the Geological Survey of Canada<sup>1-4</sup>. U and Pb were isotopically analysed on a MAT261 multicollector mass spectrometer using simultaneous ion collection of all Pb isotopes and similarly for U as outlined by Roddick *et al.*<sup>3</sup>. U blanks were all less than 1 pg for these analyses. The Pb blank was 6-15 pg for the zircons with the exception of zircon 1 (Table 1), in which the 65 pg of common Pb was entirely blank-derived, and 15-28 pg for the monazites. The chemical composition of this rock was determined by X-ray fluorescence chemical analyses (major elements, V, Ni, Nb, Zr, Y, Sr, Rb, Pb), and instrumental neutron activation analysis (other trace elements). All quoted errors in the text and tables are at the level of one sigma, whereas error ellipses on concordia plots are at the two sigma level. Results of U-Pb isotope analyses are listed in Table 1.

Nine of the ten zircon fractions analysed from sample  $\Delta 33$  plot in a triangular region on the concordia diagram (Fig. 1) bounded by lines drawn from 19.5 Myr to  $\sim 500$  and 2,300 Myr. These upper intercepts are similar to the range of Nd model ages for other Tertiary granites and metamorphic rocks from this region<sup>5-7</sup>. This pattern is typical of zircons from Tertiary leucogranites in southern Tibet<sup>8-10</sup> and can be attributed to variable amounts and ages of inherited radiogenic lead ( $\text{Pb}^*$ ) in residual zircon. Two separate zircon crystals showing no visible core or other evidence of an inherited component under the petrographic microscope were selected and analysed separ-

## Identification of inherited radiogenic Pb in monazite and its implications for U-Pb systematics

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The simplest interpretation of uranium-lead mineral ages in igneous rocks embodies the assumption that melting of pre-existing rocks leads to homogenization of the lead isotopes, thus resetting the radiometric 'clock'. Zircons often violate this assumption by only partially dissolving and preserving a component of lead 'inherited' from before the melting episode, but until now such behaviour has not been observed in monazite. Here we report U-Pb data for zircons and monazites from a Tertiary granite from the Himalayas, in which zircons and monazites contain inherited radiogenic lead. An analysis of a single entirely igneous zircon crystal



Table 1 U-Pb zircon and monazite data for  $\Delta 33$ 

| Analysis #<br>size* | wt<br>(mg) | U<br>p.p.m. | Pb†<br>p.p.m. | $^{206}\text{Pb}/$<br>$^{204}\text{Pb} \ddagger$ | Pb $\S$<br>(pg) | $^{208}\text{Pb} \dagger$<br>(%) | $^{206}\text{Pb}/^{238}\text{U} \parallel$ | $^{207}\text{Pb}/^{235}\text{U} \parallel$ | $^{207}\text{Pb}/^{206}\text{Pb} \parallel$ | $^{207}\text{Pb}/^{206}\text{Pb}(\text{Ma}) \P$ |
|---------------------|------------|-------------|---------------|--------------------------------------------------|-----------------|----------------------------------|--------------------------------------------|--------------------------------------------|---------------------------------------------|-------------------------------------------------|
| <b>Zircon</b>       |            |             |               |                                                  |                 |                                  |                                            |                                            |                                             |                                                 |
| 1. flat $n=1$       | 0.0008     | 4,629       | 12.51         | 2 <sup>o</sup>                                   | 65              | <1                               | 0.003024 (1.9)                             | 0.01928 (10.4)                             | 0.04525 (9.2)                               | 10.8 (390)                                      |
| 2. euh $n=1$        | 0.001      | 3,939       | 26.99         | 553                                              | 19              | 4.0                              | 0.007266 (0.12)                            | 0.05258 (0.23)                             | 0.05249 (0.17)                              | 306.6 (7.6)                                     |
| 3. euh $n=21$       | 0.0116     | 4,149       | 30.03         | 666                                              | 36              | 4.3                              | 0.007622 (0.12)                            | 0.06034 (0.23)                             | 0.05742 (0.16)                              | 507.7 (7.2)                                     |
| 4. euh $n=3$        | 0.0008     | 6,349       | 67.73         | 523                                              | 7               | 3.9                              | 0.01131 (1.19)                             | 0.08531 (0.25)                             | 0.05473 (0.19)                              | 401.2 (8.5)                                     |
| 5. euh < 74         | 0.0237     | 3,440       | 35.87         | 2,987                                            | 18              | 7.7                              | 0.01052 (0.10)                             | 0.09222 (0.13)                             | 0.06356 (0.05)                              | 727.1 (2.2)                                     |
| 6. euh > 74         | 0.119      | 3,407       | 41.88         | 6,822                                            | 48              | 4.4                              | 0.01287 (0.16)                             | 0.1091 (0.17)                              | 0.06148 (0.04)                              | 656.0 (1.9)                                     |
| 7. euh < 74         | 0.093      | 4,378       | 49.29         | 5,265                                            | 57              | 4.9                              | 0.01164 (0.18)                             | 0.1105 (0.19)                              | 0.06883 (0.04)                              | 893.8 (1.8)                                     |
| 8. euh > 74         | 0.064      | 3,710       | 44.27         | 5,218                                            | 35              | 5.3                              | 0.01222 (0.13)                             | 0.1279 (0.15)                              | 0.07591 (0.04)                              | 1,092.6 (1.7)                                   |
| 9. nd > 74          | 0.037      | 3,820       | 42.22         | 3,820                                            | 27              | 6.4                              | 0.01853 (0.10)                             | 0.1423 (0.12)                              | 0.05570 (0.05)                              | 440.4 (2.2)                                     |
| 10. euh > 74        | 0.0143     | 3,528       | 42.68         | 3,173                                            | 12              | 4.6                              | 0.01228 (0.11)                             | 0.1571 (0.13)                              | 0.09277 (0.05)                              | 1,483.3 (1.8)                                   |
| <b>Monazite</b>     |            |             |               |                                                  |                 |                                  |                                            |                                            |                                             |                                                 |
| 1. cyd, $n=2$       | 0.0534     | 5,447       | 65.99         | 805                                              | 78              | 75.0                             | 0.003355 (0.17)                            | 0.02056 (0.27)                             | 0.04445 (0.18)                              | <0                                              |
| 2. cdy, $n=10$      | 0.195      | 6,374       | 77.22         | 672                                              | 421             | 73.9                             | 0.003504 (0.89)                            | 0.02147 (0.93)                             | 0.04444 (0.24)                              | <0                                              |
| 3. $n=3$            | 0.073      | 7,969       | 95.43         | 856                                              | 154             | 73.5                             | 0.003509 (0.80)                            | 0.02141 (0.83)                             | 0.04425 (0.17)                              | <0                                              |
| 4.                  | 0.0491     | 6,145       | 77.79         | 690                                              | 101             | 74.7                             | 0.003542 (0.26)                            | 0.02177 (0.34)                             | 0.04457 (0.19)                              | <0                                              |
| 5. $n=25$           | 0.171      | 7,242       | 103.1         | 807                                              | 403             | 74.2                             | 0.004063 (1.0)                             | 0.02561 (1.0)                              | 0.04571 (0.19)                              | <0                                              |
| 6. $n=27$           | 0.0568     | 7,832       | 146.3         | 1,537                                            | 98              | 74.3                             | 0.005285 (0.16)                            | 0.03585 (0.20)                             | 0.04919 (0.09)                              | 156.8 (4.4)                                     |
| 7. $n=7$            | 0.184      | 8,051       | 183.5         | 1,054                                            | 1,085           | 52.0                             | 0.01202 (0.51)                             | 0.08846 (0.54)                             | 0.05336 (0.13)                              | 344.2 (5.9)                                     |
| 8. $n=4$            | 0.0305     | 8,361       | 466.4         | 5,438                                            | 67              | 62.9                             | 0.02267 (0.13)                             | 0.1723 (0.15)                              | 0.05512 (0.05)                              | 417.3 (2.0)                                     |

Weighing error 0.001 mg, except for the three zircon fractions, which weighed 0.001 mg or less, and whose weights were estimated from photomicrographs.

\* Sizes (>74) refer to length of zircons in microns; euh, euhedral with 3:1 to 6:1 aspect ratios; nd, needles with up to 8:1 aspect ratio; cdy, cloudy, zircons were all selected to be generally to extremely clear with sharp crystal faces and some contain faint visible evidence of core material.

† Radiogenic Pb.

‡ Measured ratio, corrected for spike and fractionation.

§ Total common Pb in analysis corrected for fractionation and spike.

|| Corrected for blank Pb and U, common Pb, errors quoted are 1 standard error of the mean in per cent.

¶ Corrected for blank and common Pb, errors are 1 standard error of the mean in Ma.

ately (Fig. 1, Table 1). One contained isotopic evidence for inheritance, but the other shows no such evidence and plots on concordia at 19.5 Myr with an uncertainty in the  $^{206}\text{Pb}/^{238}\text{U}$  age of 0.4 Myr. Although the special handling procedures for this sample resulted in a high blank contribution, the isotopic composition of our blank is accurately known, accounting for the relatively low uncertainty.

The observation of zircon saturation in this rock allows calculation of the peak temperature encountered during melting<sup>11,12</sup>. Table 2 gives the chemical composition of this sample and the calculated cation ratio ( $M$ ) value  $[(\text{Na} + \text{K} + 2\text{Ca})/(\text{Al} \cdot \text{Si})]$  which has been shown to embody the chemical variables which control zircon saturation in crustal magmas<sup>11</sup>. Given the compositional similarity to all other Himalayan Tertiary leucogranites<sup>13</sup>, we can conclude that this body has not experienced significant fractionation and the whole rock chemistry reflects the liquid composition. Even if significant crystals were present at peak conditions,  $M$  would not vary significantly for our purposes. Therefore the Zr concentration of 66 p.p.m. and  $M$  value of 1.2 indicates a melting temperature of  $\sim 730^\circ\text{C}$  (ref. 11). This calculation overestimates the actual temperature because of the presence of a portion of undissolved restitic zircon. Through optical and isotopic evidence of 'average' zircons in this sample, a liberal estimate of this portion is between 20 and 50%. Recalculation using 80–50% of the Zr in the whole rock yields a reduction of the calculated temperature to between 710 and 680  $^\circ\text{C}$ .

Results from the eight fractions of monazite fall on a line from an upper intercept of  $471 \pm 10$  Myr (mean square of weighted deviates (MSWD) = 19.7) to the youngest fraction, which lies above concordia at 21 Myr (Fig. 2b). Four other monazite fractions also plot above concordia between 22 and 26 Myr. That these points plot above concordia can be explained by the incorporation during crystallization of  $^{230}\text{Th}$  into the monazites and subsequent decay.  $^{230}\text{Th}$  decays to  $^{206}\text{Pb}$  with a half-life of 75,400 yr, thus producing excess (or unsupported)  $^{206}\text{Pb}$  (ref.

8). The Th/U ratio of the monazite (calculated from Pb isotope ratios) with the youngest apparent age (Table 1) and the Th/U of the whole rock (Table 2) indicate that  $\sim 10\%$  of the  $^{206}\text{Pb}$  in this fraction is from the decay of  $^{230}\text{Th}$ , given that the crystallization age is 20 Myr—virtually identical to that observed (Table 1).

The rocks that melted to produce this granite were probably older metasedimentary rocks. The excellent linear array of monazite analyses suggests that the upper intercept of  $471 \pm$

Table 2 Chemical composition of sample  $\Delta 33$ 

|                                 |       |    |       |
|---------------------------------|-------|----|-------|
| SiO <sub>2</sub>                | 73.12 | V  | <10   |
| TiO <sub>2</sub>                | 0.13  | Cr | 7     |
| Al <sub>2</sub> O <sub>3</sub>  | 15.18 | Ni | <10   |
| Fe <sub>2</sub> O <sub>3t</sub> | 1.11  | Ba | 432   |
| MnO                             | 0.02  | Nb | 9     |
| MgO                             | 0.20  | Ta | 1.0   |
| CaO                             | 0.66  | Zr | 66    |
| Na <sub>2</sub> O               | 3.44  | Hf | 2.0   |
| K <sub>2</sub> O                | 4.91  | Sr | 104   |
| P <sub>2</sub> O <sub>5</sub>   | 0.61  | Rb | 259   |
| LOI                             | 0.90  | Pb | 11    |
|                                 |       | Th | 8     |
| Total                           | 99.87 | U  | 15    |
|                                 |       | La | 15    |
| $M = 1.2$                       |       | Ce | 35    |
|                                 |       | Pr | 0.29* |
|                                 |       | Nd | 20*   |
|                                 |       | Sm | 5.5   |
|                                 |       | Eu | 0.66  |
|                                 |       | Gd | 0.24* |
|                                 |       | Tb | 0.6   |
|                                 |       | Yb | 1.2   |
|                                 |       | Lu | 0.15  |

Major elements in per cent; trace elements in p.p.m.;  $M$  equals the cation ratio  $(\text{Na} + \text{K} + 2\text{Ca})/(\text{Al} \cdot \text{Si})$ ; LOI, loss on ignition.

\* Interpolated value.

10 Myr reflects an earlier metamorphism of these source rocks, although this age may reflect averaging of discrete populations. The four essentially collinear zircon results which define the top of the triangular region yield an upper intercept of  $502 \pm 10$  Myr (MSWD = 5.1). These two results are similar to U-Pb zircon,  $^{40}\text{Ar}/^{39}\text{Ar}$  hornblende, and Rb-Sr whole rock ages obtained from orthogneisses from the Himalayas and southern Tibet (refs 14, 15 and M. S. Hubbard, personal communication). The line defined by monazite analyses clearly represents a mixture between an inherited component (with the possibility of some Pb loss) and a magmatic component at 20 Myr. The possibility that this line represents *only* lead loss at  $\sim 20$  Myr from a population of monazite with a U-Pb age of 471 Myr is precluded by the presence of excess  $^{206}\text{Pb}$  in the fractions with the youngest apparent ages. Pb loss will displace points along a chord ending at concordia, whereas incorporation of  $^{230}\text{Th}$  into a monazite during crystallization from a melt will displace points above concordia. Many workers (for example, see refs 16 and 17) have argued that the degree of concordancy is the criterion by which U-Pb monazite analyses should be judged when determining crystallization ages. Note, however, that the presence of a nearly concordant monazite fraction at about 26 Myr does not give the age of this granite, but rather it coincidentally plots near the intersection between concordia and the discordia from the upper intercept to the young fractions which contain excess  $^{206}\text{Pb}$ . The crystallization age for this granite is taken to be  $20 \pm 1$  Myr, given by the agreement between the single concordant zircon and the monazite fraction with the youngest apparent  $^{207}\text{Pb}/^{235}\text{U}$  age.

As with the presence of undissolved zircon, the identification of a restitic component in these monazites allows a second method for calculation of the temperature of melting for this granite<sup>18,19</sup>. Based on monazite solubility systematics and a whole rock light rare earth elements (LREE) concentration of 76 p.p.m. (Table 2), a maximum temperature of  $\sim 727^\circ\text{C}$  is indicated. The pattern of discordance for these monazites (highest U in oldest fractions, substantial magmatic  $^{230}\text{Th}$ ) suggests the inherited component to be  $\sim 10$ –20%. If we then take 90–80% of the total LREEs, the temperature of monazite saturation is calculated to be  $\sim 720$ –710  $^\circ\text{C}$ . This range is similar to that calculated from the zircon saturation systematics.

The U-Pb systematics of monazite from this sample confirm the conclusion of Sawka and Harrison<sup>20</sup> that residual monazite can retain an inherited Pb\* signature through a crustal melting episode. Three pieces of information are required for us to infer closure temperature significance of these data<sup>21</sup>. (1) The degree of isotopic equilibration, (2) the peak temperature of melting and (3) the duration of peak heating. The degree of equilibration is complicated by the significant growth of new monazite evidenced by the  $^{230}\text{Th}$  disequilibrium, but our view is that no more than  $\sim 10$ –20% of this monazite can be residual, implying a relatively low degree of diffusive equilibration of the restitic monazite ( $< 50\%$ ). The zircon and monazite saturation temperatures are consistent with experimental studies of water saturated or near saturated anatectic systems<sup>22,23</sup> and their internal consistency leads us to take the peak temperature to be  $710 \pm 30^\circ\text{C}$ . To explain the age contrast between leucogranite belts in the Higher and Tethyan Himalaya, Pinet and Jaupart<sup>24</sup> have proposed a thermal model which involves heat refraction from the Tibetan Slab/Tethyan sedimentary rock interface. This model predicts a duration for the melting event of about 5 Myr. Although more specific predictions must await a Pb diffusion law for monazite, these parameters imply a closure temperature of 720–750  $^\circ\text{C}$  for a cooling rate of 20  $^\circ\text{C}$  per Myr and a crystal dimension of 10 to 100  $\mu\text{m}$ . This closure temperature for Pb in monazite is significantly higher than the value of 530  $^\circ\text{C}$  suggested by Wagner *et al.*<sup>25</sup>, and somewhat higher than estimates of 650–700  $^\circ\text{C}$  (ref. 26) inferred from monazite analyses in high-grade paragneisses and other Himalayan leucogranites. Sawka and Harrison<sup>20</sup> observed that U-Pb monazite results from S-type

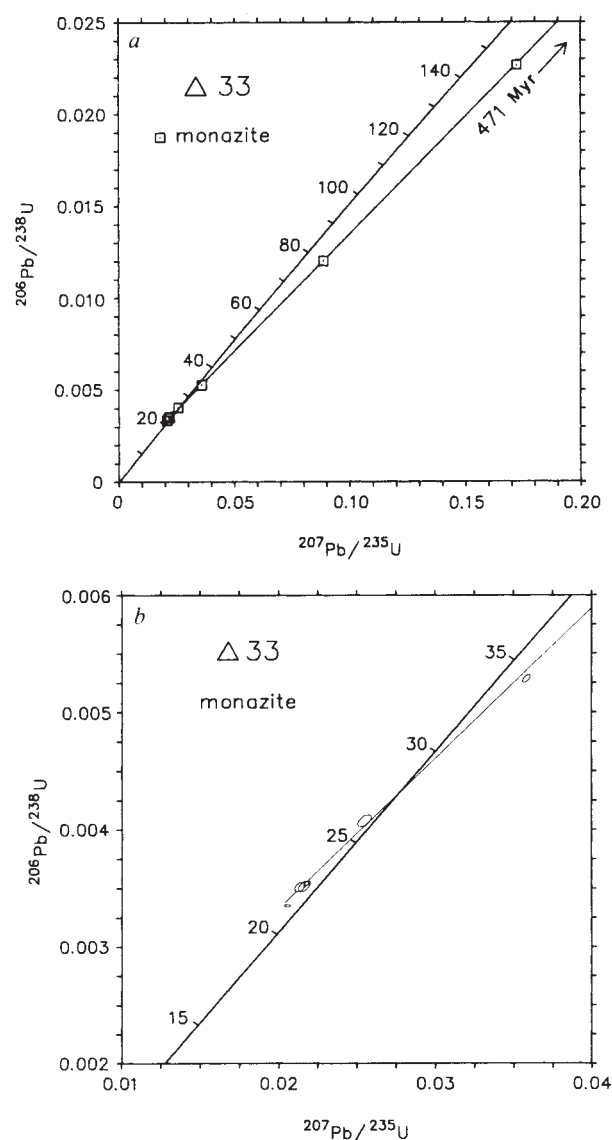


Fig. 2 Concordia diagram for monazites from  $\Delta 33$ : a, eight monazite fractions shown plot on a discordia from an upper intercept of  $471 \pm 10$  Myr to the youngest monazite fraction which plots above concordia at 21 Myr. b, Blowup of area around the monazites which contain excess  $^{206}\text{Pb}$  from the decay of  $^{230}\text{Th}$  showing two sigma error ellipses.

granites<sup>27,28</sup> are often older than independently established crystallization ages, and speculated that this might reflect Pb\* accumulation in monazite after crystallization by dehydration of LREE-rich hydrous phosphates (such as rhabdophane) during prograde metamorphism of a metasedimentary lower crust<sup>29</sup>. Although this mechanism may ultimately account for the origin of the restitic monazite, our data clearly suggest that this monazite growth did not occur during the Tertiary orogenic cycle, but during a Palaeozoic event.

The identification of inherited Pb\* in the monazites in this sample suggests caution should be used in interpreting U-Pb monazite ages from S-type granites where only one or two 'concordant' fractions were analysed, especially in magmas with high U/Th. Proper interpretation of monazite U-Pb systematics requires full understanding of its pattern of discordance based on analysis of multiple fractions of monazite.

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## Percussion marks on bone surfaces as a new diagnostic of hominid behaviour

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Since stone tool cut marks and carnivore tooth marks were first described systematically on Plio-Pleistocene archaeological bone<sup>1,2</sup>, bone surface modification has played a prominent role in interpreting early archaeological site formation and hominid behaviour<sup>3-15</sup>. Here we introduce a new class of bone surface modification, which we call percussion marks. Percussion marks were produced during experimental breakage of marrow bones, and occur as pits or grooves impressed on a bone's surface by natural protrusions on the granitic hammerstone and anvil used. Although percussion marks can superficially mimic carnivore tooth marks, they nonetheless are closely associated with hammerstone impact notches and show consistent micromorphological features which distinguish them from tooth marks and other classes of bone surface modification. Given indications of prehistoric hammerstone breakage of marrow bones<sup>1,7</sup>, an awareness of percussion marks is critical for accurately identifying the biological agents of bone modification at archaeological sites and provides a new diagnostic of carcass processing by hominids.

Fresh long bones of Thompson's and Grant's gazelle, impala and wildebeest were broken after skinning and defleshing by resting each bone horizontally on a rounded anvil surface opposite the point of intended hammerstone impact. Bones were broken so that all marrow could be efficiently removed with a 12-inch probe (see ref. 6 for details) and were subjected to no further modifying influence. 327 fragments longer than 2 cm were generated from the three main bones of 12 limbs of seven animals.

Percussion pits (roughly circular in plan) and/or grooves (displaying clear linearity) were produced on the surface of 123 specimens, or 37.6% of the sample. Marked specimens bear an average of over two pits and/or grooves, many of which become macroscopically apparent only after cleaning (boiling) and inspection under low-incidence light. To our knowledge, Free-

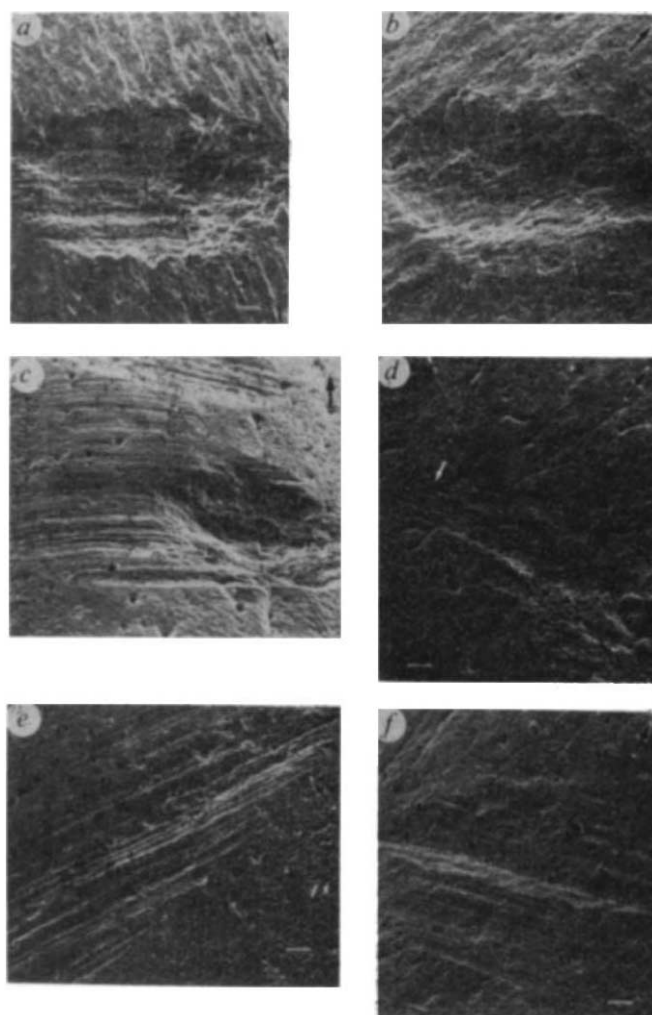


Fig. 1 *a*, Scanning electron micrograph of percussion pit with microstriations restricted mainly within pit boundaries. Uncharacteristically, microstriations were not readily visible macroscopically, but were apparent under incident-light stereomicroscope at thirty-power. *b*, Typical spotted hyena tooth pit lacking microstriations, but otherwise superficially similar to *a*. *c*, Percussion pit more typical than *a*, with microstriations emanating from it. Microstriations were conspicuous macroscopically under strong low-incidence light. *d*, Spotted hyena tooth pit with four wider, shorter and less densely packed microstriations (small arrow) than in *c*. Microstriations, which accompany tooth pits infrequently, macroscopically resemble tooth-scoring, but on a smaller scale. *e*, Percussion groove containing and flanked by microstriations. Groove is actually embedded portion of otherwise shallow patch of microstriations. *f*, Same as *e*, but groove is bevelled and its upper margin forms edge of patch of microstriations. Arrows in upper right corner indicate direction of bones' longitudinal axes. Scale bars: *a*, *c* and *d*, 0.15 mm; *b* and *e*, 0.14 mm; *f*, 0.13 mm.

man's<sup>16</sup> incidental remark on what appear to be similar modifications to several experimental bone tools is the only previous mention of such marks.

Because percussion pits (Fig. 1*a*) can mimic carnivore tooth pits (Fig. 1*b*), and percussion grooves are less easily confused macroscopically with tooth scores or other marks, we conducted a morphological comparison of 20% random samples of percussion-pitted ( $n = 21$ ) and spotted hyena tooth-pitted ( $n = 32$ ) specimens. The latter sample derives from observed episodes of spotted hyena destruction of marrow bones in natural settings<sup>5,6</sup>. After macroscopic analysis using a ten-power hand lens, pits were moulded<sup>7,15</sup> and examined under a light microscope at 30 magnifications.

**Table 1** Incidence and density of microstriations associated with pits in a 20% random sample of percussion-pitted specimens\* and spotted hyena tooth-pitted specimens

|                                              | Percussion      | Carnivore       |
|----------------------------------------------|-----------------|-----------------|
| Association between pits and microstriations |                 |                 |
| Macroscopic:                                 |                 |                 |
| Total number of specimens                    | 21              | 32              |
| Number (%) of pits with microstriations      | 18 (85.7)       | 0 (0)           |
| Microscopic:                                 |                 |                 |
| Total number of specimens†                   | 18              | 26              |
| Number (%) of pits with microstriations      | 17 (94.4)       | 5 (19.2)        |
| Density of microstriations                   |                 |                 |
| Total number of specimens                    | 15‡             | 5               |
| Number of microstriations per mm: range      | 18–36           | 8–16            |
| Mean $\pm$ 1 s.d.                            | 26.8 $\pm$ 4.66 | 12.0 $\pm$ 2.80 |

Macroscopic search for microstriations was made on actual bone specimen under strong, low-incidence light. Microscopic search was made with a stereomicroscope at 30 power on latex ('Xantopren') moulds of pitted bone surface. Density measurements were made on latex moulds at 30 magnifications, using a micrometer disk with a 10-mm scale divided into 100 parts. The density of carnivore-produced microstriations is significantly less than those produced by percussion: Mann-Whitney-Wilcoxon  $W$  test,  $Z = -3.27$ ,  $P < 0.001$  (two-tailed).

\* Excludes the 20 percussion-marked specimens bearing only grooves.

† Excludes specimens that could not be moulded for microscopic analysis because pit was on the edge of bone specimen.

‡ Excludes two moulded specimens with patch of microstriations too narrow to yield an accurate estimate of density.

Most of the sample of percussion pits are associated with patches of microstriations that are usually visible macroscopically (Table 1), and occur either in (Fig. 1a) or emanating from (Fig. 1c) a pit, or within several centimeters of it. Microstriations (usually  $< 1$  cm long) are produced by slight slippage of the hammer, or of the bone on the anvil, on impact. Conversely, unequivocal microstriations were not macroscopically visible on any of the tooth-pitted specimens (Fig. 1b) and were found to be present on microscopic inspection on fewer than 20% of these (Table 1). The microstriations produced by spotted hyenas (Fig. 1d), however, are distinctly broader, being produced perhaps by irregularities of, or foreign particles on, a tooth's occlusal surface. As a result, tooth microstriations occur in patches significantly less dense than those produced by percussion (Table 1). Microstriations also consistently accompany percussion grooves (Fig. 1e and f) which result from greater slippage of the bone upon impact. Except for being more deeply embedded and generally longer ( $> 2$  cm), patches of microstriations associated with percussion grooves are similar to those that accompany percussion pits.

Constituent striae of a patch of microstriations are usually straight and parallel (70.6% of the random sample), with a transverse ( $\pm 15^\circ$ ) orientation to the bone's long axis (76.5%) (Fig. 2a, also Fig. 1a, c, e and f). Percussion microstriations differ from stone tool-cut marks (Fig. 2b; refs 1–3, 7, 10, 15) in being shallower, narrower, and usually shorter and occurring in dense unidirectional patches. They are also distinct from scraping marks (inflicted while removing periosteum before breakage; Fig. 2c), which are substantially longer and are oriented within  $15^\circ$  of parallel to the bone's long axis<sup>4,15</sup>, and from trample marks (Fig. 2d), which are usually longer and lack uniform and/or predictable directionality<sup>3,17</sup>. These distinctions render the macroscopic identification of percussion marks unambiguous in most cases, especially if their predictable anatomical location is considered<sup>18</sup>.

In our experimental sample, percussion marks usually accom-

pany more conspicuous features of hammerstone-on-anvil impact. In particular, impact events preferentially produced percussion marks in direct physical association with hammerstone impact notches (Table 2). Further, 79.7% of all marked specimens bear at least one mark within 5 mm of fracture edges originally tangential to the hammerstone or anvil surface.

The incidence and micromorphology of percussion marks in archaeological bone assemblages is likely to vary with breakage technique, depending especially on the surface texture of the hammerstone and anvil used, but we have replicated percussion marks using a sandstone hammerstone and anvil considerably more fine-grained than granite. Percussion marks therefore have a high probability of occurring in archaeological long bone assemblages fractured by prehistoric hominids using stone with a rough surface (for example, the quartzite and basalt used during Early Pleistocene times at Olduvai Gorge, Tanzania<sup>19</sup>).

The tooth mark mimicking of percussion marks may confound archaeological interpretations based on the occurrence of tooth-marked and tool-marked bone. These include the role of carnivores versus hominids in accumulating and modifying an archaeological bone assemblage<sup>4,6,7,9</sup>, and the mode of carcass acquisition (hunting versus scavenging) and type of foraging strategy practised by early hominids<sup>2,4,5,9,14,20</sup>. Given the potentially common occurrence of percussion marks on archaeological bone suggested by our experimental results, their omission from the range of surface marks currently sought by fossil analysts could skew such interpretations significantly.

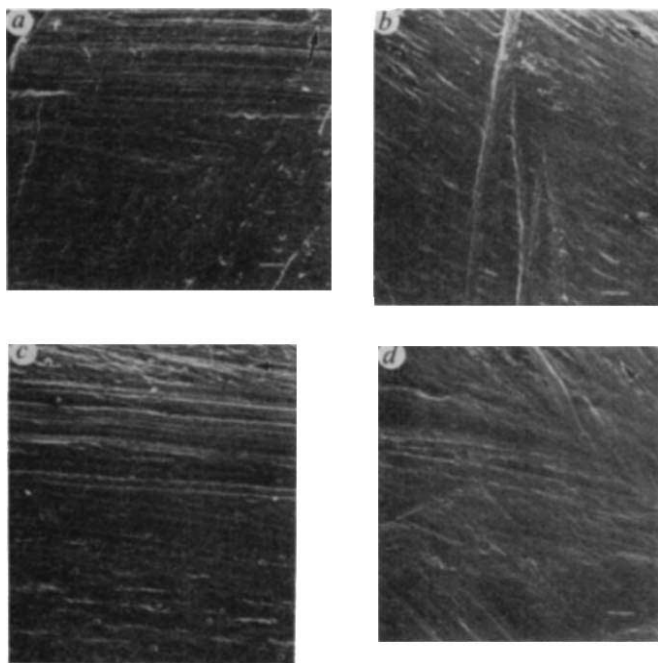
Percussion marks also provide a new diagnostic of hominid involvement with bone. Although physical mechanisms of impact fracture (as in rock falls) might produce percussion mark mimicking, these should be anatomically unpatterned and expected only in high-energy geological contexts. Therefore, percussion-marked bone in most contexts should clearly diagnose hominid activity, and prove particularly useful where traditional, well established evidence of hominid butchery (such as cut marks) or hominid presence (such as stone tools) is absent. For example, percussion marks provide a test for those claims of an early (pre-Clovis) human presence in the New World that are currently based on taphonomically ambiguous patterns of bone fracture, or on tools allegedly shaped from bone<sup>4,21–29</sup>. Further, percussion marks can be used to search for possible

**Table 2** The association between percussion-marking and episodes of hammerstone impact\*

|                                              | With percussion mark<br><i>n</i> (%) | Without percussion mark<br><i>n</i> (%) |
|----------------------------------------------|--------------------------------------|-----------------------------------------|
| <i>a</i>                                     |                                      |                                         |
| Impact-notched specimens ( <i>n</i> = 90)    | 58 (64.4)                            | 32 (35.6)                               |
| Non-notched specimens ( <i>n</i> = 237)      | 65 (27.4)                            | 172 (72.6)                              |
| $(\chi^2 = 37.9$ , d.f. = 1, $p < 0.001$ )   |                                      |                                         |
|                                              | With impact notch<br><i>n</i> (%)    | Without impact notch<br><i>n</i> (%)    |
| <i>b</i>                                     |                                      |                                         |
| Percussion-marked specimens ( <i>n</i> = 78) | 55 (70.5)                            | 23 (29.5)                               |
| Unmarked specimens ( <i>n</i> = 65)          | 23 (35.4)                            | 42 (64.6)                               |
| $(\chi^2 = 17.8$ , d.f. = 1, $p < 0.001$ )   |                                      |                                         |

\* Measured by the presence of hammerstone impact notches (namely, broad arcuate indentations of the bone's fracture edge at the zone of hammerstone impact; see refs 6, 21 and 30). *a*, Occurrence of percussion marks on impact-notched specimens, either directly at and/or directly opposite the notch. *b*, Occurrence of impact notches on percussion-marked specimens. Includes only specimens preserving more than  $90^\circ$  of the bone shaft's circumference, so that those marked only by anvil (bottom face of bone) could be inspected for hammerstone impact notch on opposite (top) face.





**Fig. 2** *a*, Isolated patch of percussion microstriations showing characteristic straight and parallel geometry of constituent striae, and their transverse orientation to bone's longitudinal axis (arrow) seen also in Fig. 1*a*, *c*, *e* and *f*. *b*, Cut marks inflicted experimentally by stone (chert) flake. Individual cut marks are longer, thicker and deeper than percussion microstriations, and occur in less dense patch that lacks strict unidirectionality and a transverse orientation to bone's longitudinal axis (arrow). See also photos in refs 2 and 15. *c*, Small section of two overlapping patches of scraping marks inflicted by stone (chert) flake while experimentally removing periosteum before hammerstone breakage. Constituent microstriae of each patch are straight, parallel, shallow and dense, as in *a*, but are oriented essentially parallel to bone's longitudinal axis (arrow). Scraping produced distinct shaving between striae which was not seen in *a*. *d*, Trample marks produced experimentally by treading on bone with rubber-soled shoes over coarse, poorly sorted sand substrate for two minutes (see also photos in ref. 3). Note typical multidirectionality and uneven thickness and depth of microstriae. The arrow indicates the direction of the bone's longitudinal axis.

Scale bars: *a* and *c*, 0.23 mm; *b*, 0.26 mm; *d*, 0.14 mm.

hominid involvement with animal carcasses before the apparent advent of flaked-stone technology ~2.4 million years ago, when possibly unmodified cobbles were used to process marrow bones of carcasses found defleshed (compare ref. 5). If so, percussion marks could substantially extend the time depth of archaeological evidence for human behavioural evolution.

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## Early hominid diets from quantitative image analysis of dental microwear

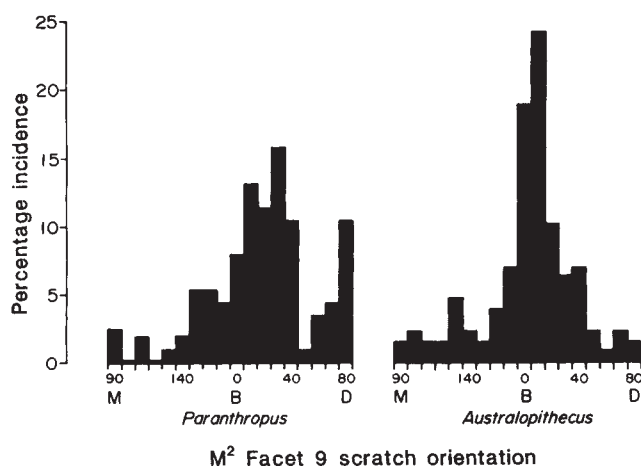
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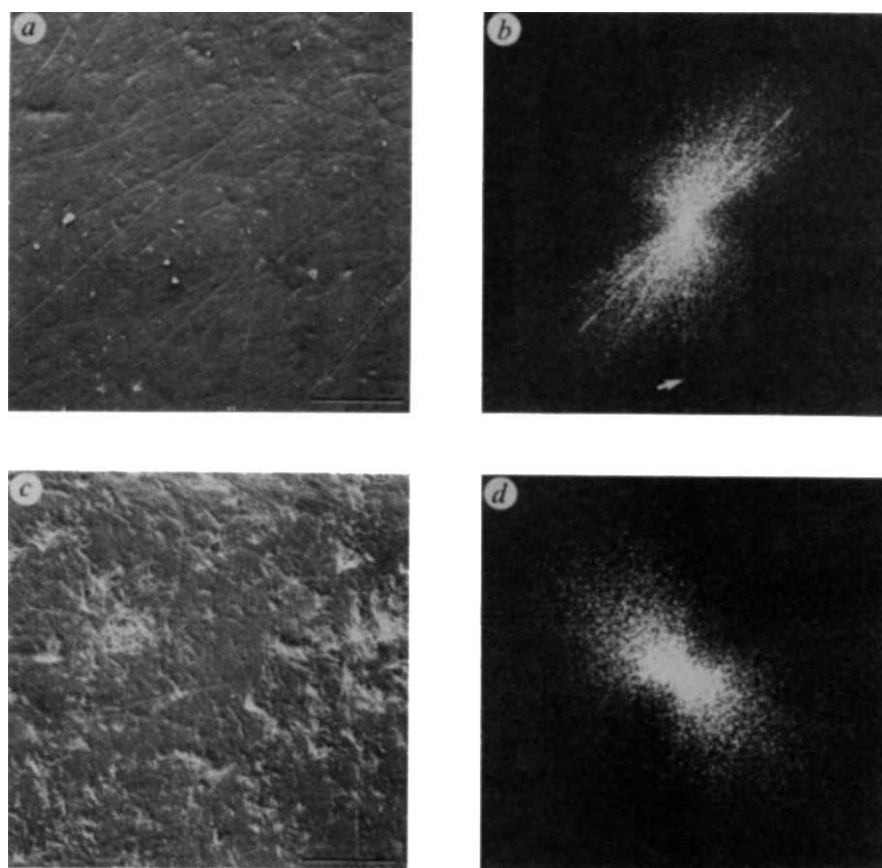
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The dietary habits of the early hominids *Australopithecus* and *Paranthropus* have long been debated. Robinson<sup>1</sup> argued that the two species differed in the proportions of meat and vegetables consumed. More recently it has been suggested<sup>2,3</sup> that *Paranthropus*, with its presumably larger body size, simply processed greater amounts of the same foods eaten by *Australopithecus* to maintain 'functional equivalence'. Microscopic dental wear patterns are related to the dietary habits of extant mammals, and quantification of these patterns is useful in distinguishing among primates with different diets<sup>4–8</sup>. Nevertheless, few attempts have been made to use microwear in the reconstruction of early hominid diets<sup>3,9–11</sup>, and only very recently has the quantification of such data been initiated<sup>12,13</sup>. While microwear fabrics can be reduced to individual elements (for example, scratches and pits), there is some disagreement over exactly how they should be defined and measured<sup>5,6,12</sup>. Fourier transforms have been applied successfully in the study of a variety of physical and biological patterns<sup>14–16</sup>, and recently they have been used to characterize and distinguish different tooth wear patterns more objectively<sup>17</sup>. Here we report the first combined use of image processing and other quantitative techniques to analyse the dental microwear of early hominids. Our results suggest that *Paranthropus* ate substantially more hard food items than *Australopithecus*.

The present sample comprised epoxy replicas of maxillary second molars of five specimens of *Australopithecus* from Sterkfontein (Sts 28, Sts 30, Sts 31, Sts 52, TM 1511) and five *Paranthropus* specimens from Swartkrans and Kromdraai (SK 13, SK 16, SK 42, SK 48, TM 1517). Enamel wear on protocone facet 9 (ref. 18) was examined by scanning electron microscopy (SEM) at 10 kV in the secondary mode; each facet was micrographed at what was judged to be its center. SEM collector-specimen geometry is an important consideration in an analysis of this type, because wear features orientated parallel to the collector-specimen axis tend to appear less distinct than those



**Fig. 1** Histograms of occlusal wear scratch orientation on *Paranthropus* and *Australopithecus* molars. M, mesial; B, buccolingual crown axis; D, distal. Scratches with an angle between 0° (straight buccolingual) and 90° (straight mesiodistal), that is, those between B and D, possess a distobuccal-mesiolingual orientation. Scratches between 90° and 180°, that is, those between M and D, possess a mesiobuccal-distolingual orientation. Note the relatively platykurtic nature of the *Paranthropus* distribution. A Kolmogorov-Smirnov test reveals a significant difference between the sample distributions (observed  $D = 0.265$ , expected  $D = 0.175$ ) at the  $P < 0.05$  level, and a  $G$ -test indicates a significant difference between them ( $G_{adj} = 31.5457$ ) at the  $P < 0.001$  level; a Watson and Williams test reveals no significant difference in angular variance.



**Fig. 2** Scanning electron micrographs of occlusal wear fabrics and power spectra produced by Fourier transformation for specimens of *Australopithecus* (Sts 28) (a, b) and *Paranthropus* (SK 42) (c, d). Scale bars on micrographs = 100  $\mu\text{m}$ . Solid vertical line on b (arrowed) is an artefact of the Fourier transform.

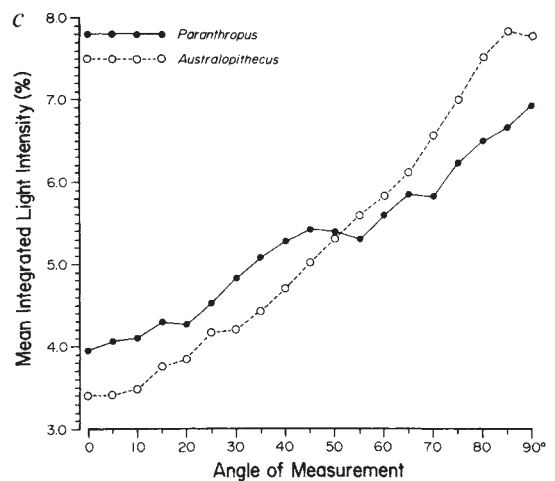
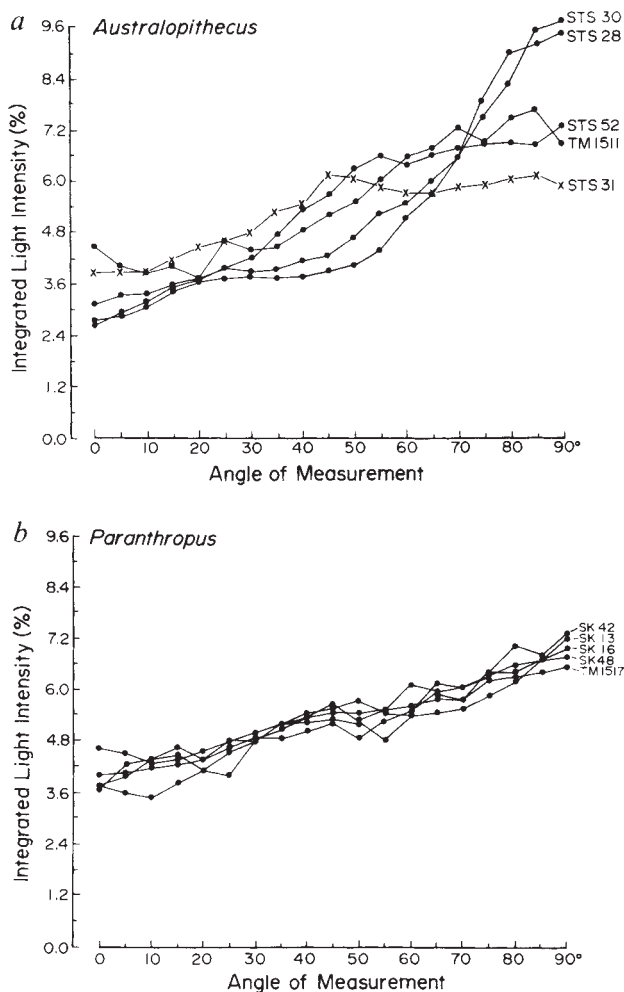
with other alignments. However, data obtained from micrographs recorded at 90° to one another reveal no significant difference in observed distributions of wear scratch orientation<sup>19</sup>, although individual feature sizes may differ according to specimen orientation. Here the potential problem of secondary electron collector bias is averted by virtue of the fact that each facet was orientated nearly perpendicular to the electron beam (that is, with minimal tilt), and specimens were orientated at random (albeit along similar axes) with respect to the collector. The numbers of scratches and pits per  $\text{mm}^2$ , scratch orientation and individual feature dimensions were recorded following the methods used elsewhere<sup>12</sup>.

Image processing was performed by digitizing the negative of each micrograph (magnification  $\times 200$ ) on a microdensitometer which gave a  $512^2$  pixel matrix of optical density

values. The matrix was analysed using the 'SPIDER' software system<sup>20</sup> so as to obtain a power spectrum by computation of a numerical Fourier transform<sup>21</sup>. To facilitate comparisons of the power spectra, integrated light intensity (ILI) was calculated at 5° intervals from the centre to the outer edge through 180° for each power spectrum; the resultant array was then aligned so that the largest ILI value corresponded to 90°. ILI values from 0° to 90° were summed for each micrograph, and the individual values were expressed as percentages of the summed total. The resultant plots of percentage ILI versus degrees of image rotation are analysed here.

The incidence of occlusal pitting is significantly higher on *Paranthropus* (49.1%) than on *Australopithecus* (29.2%) crowns ( $\chi^2 = 8.31$ ;  $P < 0.005$ ). At the same time, pit dimensions recorded for *Paranthropus* (length  $\bar{X} = 12.18 \mu\text{m}$ , s.d. = 1.07; width  $\bar{X} =$





**Fig. 3** Bivariate plots of integrated light intensity (ILI) measured radially from the centres of the power spectra versus angle of measurement in degrees for (a) five specimens of *Australopithecus*, (b) five specimens of *Paranthropus*, and (c) the *Australopithecus* and *Paranthropus* sample means. The ILI values were calculated at 5° intervals through 180° for each power spectrum (ILI values are not calculated through 360° because the power spectrum of a microwear field is symmetrical so that one half of the spectrum is the reverse mirror-image of the other). When ILI values are plotted against degrees of rotation through 180° the resultant curve commonly takes the form of a slightly asymmetrical bell, and because the form of the major peak (as dictated by the principal wear features) is of interest here, it can be legitimately smoothed by analysis of one half (90°) of the 180° scatterplot.

8.54, s.d. = 1.30) and *Australopithecus* (length  $\bar{X}$  = 8.41, s.d. = 1.52; width  $\bar{X}$  = 5.00, s.d. = 0.91) differ significantly (length  $t$  = 4.54,  $P$  < 0.002; width  $t$  = 4.99,  $P$  < 0.002). Scratches are also significantly broader on *Paranthropus* molars ( $\bar{X}$  = 1.94, s.d. = 0.55 versus  $\bar{X}$  = 0.98, s.d. = 0.07;  $t$  = 3.87,  $P$  < 0.005), with the result that pooled feature width is significantly greater for the *Paranthropus* sample ( $\bar{X}$  = 5.21, s.d. = 0.80 versus  $\bar{X}$  = 2.60, s.d. = 0.88;  $t$  = 4.91,  $P$  < 0.002).

Some 78.1% of scratches on *Paranthropus* and 73.4% on *Australopithecus* crowns display a distobuccal-mesiolingual orientation. The sample distributions (Fig. 1) differ significantly according to a  $G$ -test and a Kolmogorov-Smirnov test, which will be conservative for grouped data of this sort<sup>22</sup>, although a Watson and Williams test<sup>23</sup> indicates that there is no significant difference in angular variance. As the angular deviation is similar, the samples would appear to differ principally in mean direction.

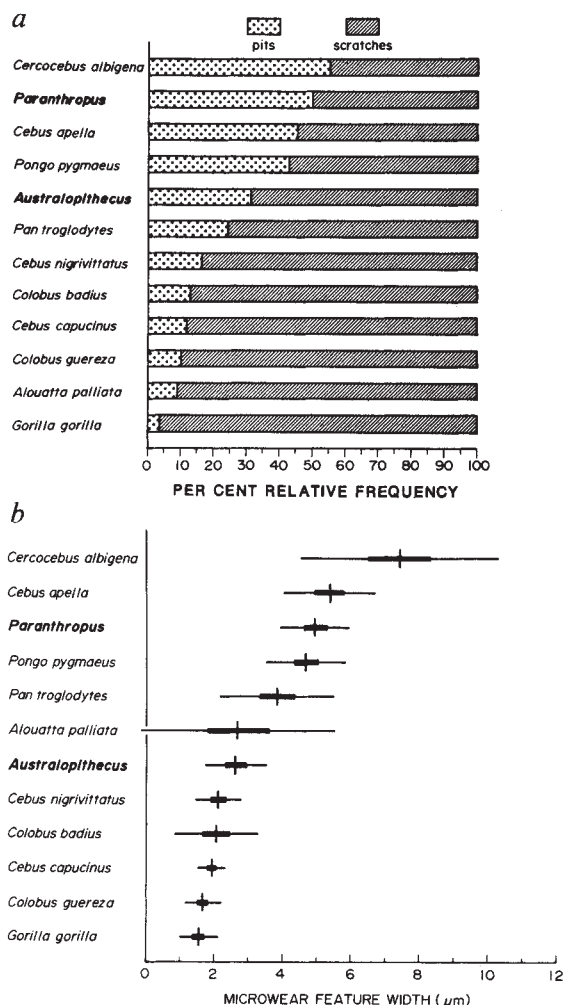
Examples of *Australopithecus* and *Paranthropus* wear surfaces and the corresponding power spectra are shown in Fig. 2. The power spectrum for the *Australopithecus* specimen reveals bands of light concentrated at right angles to the predominant long axes of the wear scratches; the *Paranthropus* spectrum is more diffuse. Plots of the ILI versus angle of measurement for these and the other specimens comprising the present samples are depicted in Fig. 3. The *Paranthropus* sample displays markedly less internal variability, and were the plots extended to a full 180° by mirror-imaging, those for *Paranthropus* specimens would be seen to be noticeably more platykurtic than those of four of the five *Australopithecus* specimens. The comparatively flat profile of Sts 31 relates primarily to the diffuse array of scratch orientations, as this wear fabric possesses comparatively few

pits. By contrast, the diffuse power spectra and concomitantly flat bivariate plots of *Paranthropus* specimens (Fig. 3) appear to be related primarily to the high frequencies of occlusal pits.

Application of randomization tests for absolute and squared differences of means of radial light intensity versus degrees of rotation (Fig. 3c) reveal that of the 126 possible ways in which the ten specimens could be combined to form two groups of five specimens each, eight (6.35%) produce greater absolute differences than we observed, and nine (7.14%) give greater squared differences than obtained for the present sample configuration. Randomization tests are very conservative<sup>22</sup>, and the observed mean differences are close to the 0.05 probability limit. Moreover, all specimen combinations that gave higher absolute and squared differences of means involved the removal of Sts 31 from the *Australopithecus* sample.

A dietary interpretation of the microwear differences between *Australopithecus* and *Paranthropus* is most reasonably based upon appropriate primate models. Thus, the distinctive power spectra and resultant bivariate arrays obtained for *Australopithecus* and *Paranthropus* are similar to, albeit less pronounced than the spectral differences recorded<sup>17</sup> for *Ateles geoffroyi* and *Chiropotes satanas* respectively. *A. geoffroyi*, which evinces fewer occlusal pits than *C. satanas*, consumes principally mature fleshy fruits, whereas the latter feeds primarily upon seeds that are encased within a tough or hard exocarp<sup>24</sup>.

Likewise, the occlusal pitting frequency recorded for *Paranthropus* (Fig. 4a) is most similar to the incidences of living species (for example, *Cercocebus albigena*, *Cebus apella* and *Pongo pygmaeus*) that feed on hard food items such as date palm seeds, palm nuts and bark<sup>25-27</sup>. The *Australopithecus* incidence falls between those of *Pongo* and *Pan troglodytes*,



**Fig. 4** Comparisons of (a) percentage incidences of microwear pitting and (b) pooled microwear feature widths recorded for  $M^2$  facet 9 of *Australopithecus*, *Paranthropus* and extant primate taxa of known dietary habits. Measurements on the fossils revealed length-width ratios for pits below 4:1 in almost all instances. Pitting frequencies for extant taxa calculated from unpublished data by courtesy of M. F. Teaford with use of a length-width ratio less than or equal to 4:1 to define pit category; these data are comparable to the early hominid sample data, whereas the incidences recorded by Teaford and Walker<sup>6</sup> are based on a ratio of 10:1 and are therefore comparatively high. Pooled microwear feature width was computed by summing individual pit and scratch values without adjustment for relative frequency of the features<sup>6,12</sup>. Data for extant primate taxa supplied courtesy of M. F. Teaford with individual specimen averages used to compute sample statistics (these values differ from those published by Teaford and Walker<sup>6</sup>). Vertical line = mean; horizontal line = 1 s.d. on either side of mean; horizontal bar = 1 s.e. on either side of mean.

whose frugivorous diet rarely (if ever) includes such hard items<sup>28,29</sup>. Similarly, comparisons of pooled feature widths (Fig. 4b) suggest that the diet of *Paranthropus* consisted of comparatively hard objects, whereas softer fruits and/or foliage comprised more of the dietary staples of *Australopithecus*.

The quantitative evidence from permanent molar microwear indicates that the diets of *Australopithecus* and *Paranthropus* were qualitatively dissimilar. *Paranthropus* wear fabrics, which evince significantly more pits, significantly broader features, and which give noticeably more diffuse power spectra, are strongly suggestive of a diet that comprised more hard food items. The pattern displayed by *Australopithecus* differs in the direction of living species that subsist more on leaves and/or fleshy fruits. The substantial differences in the masticatory morphology of

*Australopithecus* and *Paranthropus* were probably related to these qualitative differences in their diets.

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## A new Lower Carboniferous tetrapod locality in Iowa

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The earliest tetrapods known are from two or three Upper Devonian<sup>1-3</sup> and some 20 Lower Carboniferous localities in Scotland<sup>4</sup> and North America<sup>5-8</sup>. Most sites yield few and fragmentary specimens; well-preserved and even partially articulated material is exceedingly rare. This report discusses a middle Lower Carboniferous site rich in amphibian and fish remains discovered near Delta, south-east Iowa, and represents the first Lower Carboniferous tetrapod locality found in mid-continental North America. The bones occur within collapse-structures or depressions, and appear to represent a fresh- or brackish-water pond fauna. The Delta site contains the oldest well-preserved tetrapod fauna in North America, and one of the oldest in the world. Several hundred tetrapod fossils have been collected to date, with excavation somewhat more than half completed. Specimens range from isolated bones to articulated, nearly complete skeletons of at least two apparently new amphibian species. This material will make an important contribution to knowledge of the morphology and interrelationships of early tetrapods.



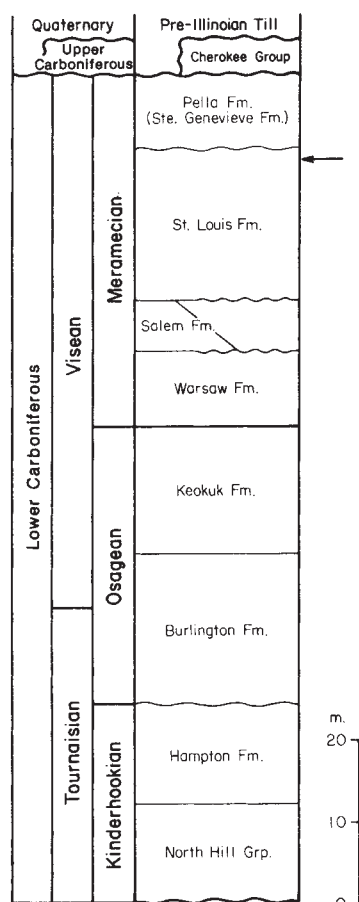


Fig. 1 General Lower Carboniferous stratigraphy of southeast Iowa. Arrow shows stratigraphic position of Delta site.

The Delta locality comprises the fills of two adjacent funnel-shaped depressions or collapse structures that formed in the upper St Louis Formation before development of the regional unconformity separating the St Louis from the Pella (=Ste Genevieve) Formation (Fig. 1). Biostratigraphic relations of the upper St Louis and basal Pella indicate a middle Late Viséan (Asbian; V3b) age for the fills<sup>9-11</sup> (~335 Myr old). Rocks exposed at the site, a disused limestone quarry, can be discussed in terms of five units<sup>11</sup> (Fig. 2). Unit A, 9-m thick, is a sequence of interbedded limestone, sandstone and shale which records the marine to non-marine transition associated with regional offlap of the St Louis sea. The lower 6 m represents a normal-marine to restricted-marine succession composed of fossiliferous bioturbated lime wackestones-packstones grading upward to sparsely fossiliferous laminated wackestones-mudstones. The fauna includes corals, echinoderms, brachiopods, molluscs, bryozoans, foraminifera, trilobites, ostracods and conodonts. The upper 3 m of unit A, composed of shale and fractured to conglomeratic lime mudstone, was apparently deposited in non-marine aquatic environments. Characteristic stenohaline faunal elements are absent; the fossil assemblage includes possible plant moulds, root moulds, ostracods, small 'spirorbid' snails, xenacanth teeth, elasmobranch, palaeoniscoid, and acanthodian scales, and other fragmentary vertebrate remains<sup>11</sup>.

The depressions truncate a portion of the upper St Louis section and display collapse of surrounding strata. Their development may have been aided by dissolution of bedded gypsum at depth in the lower St Louis section. Solutional pitting of the depression walls suggests subaerial exposure. Basal portions of the fills (Unit B, Fig. 2) contain angular to subrounded and pitted, pebble- to boulder-sized clasts derived from Unit A,

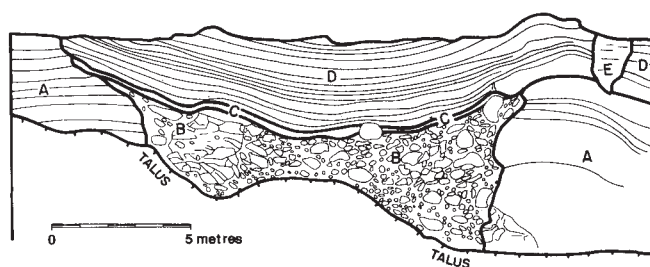


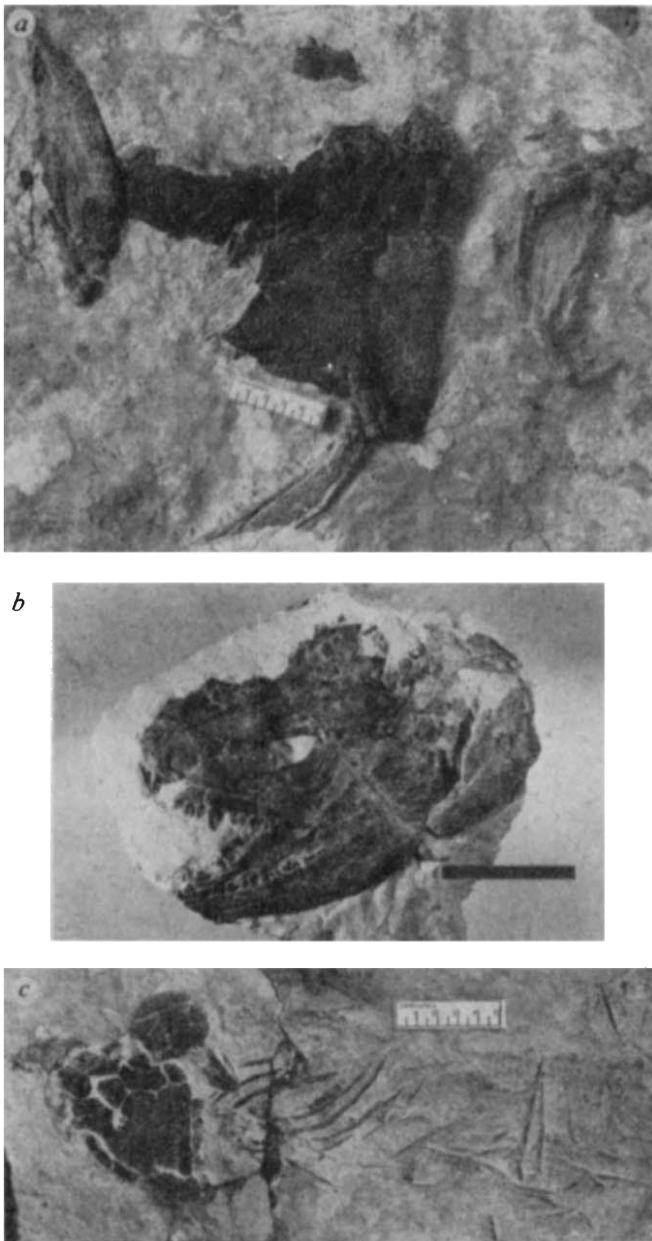
Fig. 2 Stratigraphy of the Delta site. Units are as follows. Unit A; interbedded limestone, sandstone and shale. Unit B, material derived from Unit A in a green shale matrix with scattered tetrapod bones. Unit C, main bone bed. Unit D; ostracod lime mudstones. Unit E, sandstone possibly of Pennsylvanian age.

in a green shale matrix with scattered tetrapod bones. The main bone bed (Unit C, Fig. 2) is up to 50-cm thick and locally overlaps parts of Unit A at the fill margins. The bone bed is dominated by layers and lenses of limestone conglomerate containing subangular to rounded, sand- to pebble-sized clasts of upper Unit A limestone, in a lime mud matrix. The bone-bearing conglomerates accumulated via periodic transport into the depression of debris from the surrounding St Louis surface. The conglomerates are interbedded and intergrade laterally with thin, laminated brownish-green calcareous shales, deposited under quieter subaqueous conditions. Both conglomerates and shales are rich in tetrapod bone; fish material is moderately abundant.

Unit D (Fig. 2) sharply overlies the bone bed and overlaps the upper surface of Unit A. It is dominated by dense, well-bedded, faintly-laminated ostracod lime mudstones with minor thin shales. A basal green shale contains abundant fish scales and phosphatized coprolites as well as some tetrapod bone. Rare arthropod remains recovered from this shale include the oldest acanthopterid myriapods known (B. Beall, personal communication). The lime mudstones yield a fauna virtually identical to that noted in the upper part of Unit A. Large rhizodont crossopterygian scales are abundant near the base, and tetrapod bones occur in the basal 1 m (Fig. 3a). Unit D was deposited as base levels rose, resulting in complete flooding of the depressions and surrounding areas. Its even bedding and fine-grained character and the absence of normal-marine stenohaline faunal elements suggest quiet lagoonal or lacustrine conditions, probably brackish to freshwater. It is laminated with no evidence of burrowing, suggesting deposition within an oxygen-stratified enclosed or semi-enclosed body of water.

At least two amphibian and eight fish species are present. Amphibians include a colosteid temnospondyl and a species informally referred to here as a 'proto-anthracosaur'. The colosteid (Fig. 3a) is identified by overall skull shape and proportions, the disparity in size between maxillary and (larger) dentary teeth, and the presence of markedly enlarged premaxillary fangs. It is likely to be distinct from all other colosteids, as it appears to lack the elongate Meckelian fenestra of *Greerpeton*<sup>8</sup> and the single row of coronoid teeth of *Colosteus*<sup>12</sup> and to differ from *Pholidogaster* in the shape of the postparietal and extent of the maxilla<sup>13</sup>. This is the earliest temnospondyl known from North America, and one of the oldest in the world. Colosteids have been described from two Viséan localities in the Midlothian Coalfield (Scotland)<sup>12</sup>, and the possibly Namurian Greer and Hinton localities<sup>8</sup> and the Westphalian D of Linton<sup>13</sup> (USA).

The proto-anthracosaur (Fig. 3b) is identified by cranial characters that include a tabular horn of anthracosaur type<sup>14</sup>. The skull table retains an intertemporal, as in anthracosaurs (the primitive tetrapod condition), but differs from that of anthracosaurs in lacking a tabular-parietal contact. That contact



**Fig. 3** Fossil vertebrates from the Delta site. *a*, Colosteid skull in dorsal view, anterior at top. The left cheek and maxilla have separated from the rest of the skull. The positions of the left and right lower jaws are reversed, and the right lower jaw has separated into dentary (lying just behind the skull) and post-dentary portions (on the left side of the skull). Scale bar = 5 cm. *b*, Proto-anthracosaur in dorso-lateral view, anterior to left. Scale bar = 10 cm. *c*, Lungfish skull and anterior portion of postcranium in dorsal view, anterior to left. Scale bar, 5 cm.

is characteristic of anthracosaurs and a derived condition for tetrapods. Its absence in the proto-anthracosaur is primitive and resembles the condition in Palaeostegalia (= *Crassigyrinus*<sup>14</sup>). The proto-anthracosaur lacks such palaeostegalian autapomorphies as a deep cheek below the orbit and a minute forelimb<sup>14</sup> as well as, apparently, some primitive palaeostegalian features including the retention of a preoperculum. It may thus occupy a cladistic position between palaeostegalians and anthracosaurs. The trunk vertebrae are schizomeres (intercentra massive, unpaired U-shaped elements, pleurocentra slender, paired half-rings reaching far ventrally<sup>15</sup>), a condition hitherto reported

only in temnospondyls<sup>12,15</sup>. Many of the ribs have large uncinate processes, contradicting the suggestion that this is a shared derived character of ichthyostegalian, temnospondyls and microsaur<sup>16</sup>. If the considerably older *Ichthyostega* is considered as a sister-group of all other tetrapods such ribs are primitive for tetrapods. The proto-anthracosaur is approximately coeval with the earliest reported anthracosaurs, undescribed specimens from the Scottish Brigantian (P2)<sup>2,3</sup>. It is represented by numerous disarticulated bones and several more or less complete articulated skeletons, possibly including one or more complete and articulated foot skeleton. This material promises to yield information of great importance for the study of early tetrapod relationships in general, and anthracosaur (and possibly amniote) evolution in particular.

The fish fauna includes at least one species each of lungfish, rhizodont and rhipidistian crossopterygians, palaeoniscoid, acanthodian, xenacanth and petalodont. Lungfish are represented by a nearly complete skeleton with skull, which is most similar to that of *Ctenodus* (Fig. 3c). Rhizodont crossopterygian material includes numerous scales, dermal girdle bones (some in articulation), individual cranial bones and partial skulls. Rhizodont remains are rarely found in association, and the group is consequently poorly known<sup>17</sup>. One nearly complete (although crushed) skeleton of an osteolepiform crossopterygian has been recovered. Palaeoniscoids are represented by scales and fragmentary skeletons. Acanthodian specimens include a number of spines and possibly pinnal plates of *Gyracanthus*, plus at least one partial body fossil. Xenacanth remains include a number of calcified cranial cartilage elements, one crushed skull, and separated teeth. Petalodonts are represented only by teeth.

Vertebrate faunal composition at Delta is broadly similar to that of other aquatic Lower Carboniferous sites, both North American and Scottish. A complete census will require further preparation and study; but the Delta tetrapod fauna is likely to prove more diverse than indicated in this preliminary survey. In terms of abundance and quality of preservation, particularly of articulated tetrapod specimens, the Delta site ranks among the most important palaeontological finds in many years.

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# Substrate choices made by marine larvae settling in still water and in a flume flow

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Larvae of several species of invertebrates that live just below the surface of the seabed (infaunal species) are known to make active and consistent choices among sedimentary habitats; generally they choose the sediments most similar to those occurring in the habitats where the adult stages live in the field<sup>1-5</sup>. But, previous experiments designed to investigate habitat selection by such larvae were conducted almost entirely in still water and never under realistic flow conditions. Therefore, although larvae are able to actively select settlement sites, it is not known whether these choices are exercised in natural field flows<sup>6</sup>. We report here that the larvae of the infaunal polychaete, *Capitella* sp. I and the infaunal bivalve *Mercenaria mercenaria*, actively choose appropriate substrates in still water, but the species differ in their selection capabilities under controlled, realistic and defined flow conditions in the laboratory.

The experiments were designed to determine the relative importance of active habitat selection versus passive deposition in still water and in a relatively slow, turbulent flow for two infaunal species which vary widely in life-history characteristics. *Capitella* sp. I (ref. 6) is a subsurface, deposit-feeder, commonly occurring in disturbed, organic-rich, muddy (high silt plus clay) habitats<sup>7,8</sup>, and having relatively large (450–500- $\mu$ m length), lecithotrophic larvae that can remain in the plankton for hours to days when uncued, but that can settle within a few minutes of hatching when offered an appropriate substrate (for example, organic-rich muds<sup>9,10</sup>). *Mercenaria mercenaria* (Linn.) is a filter-feeder, generally occurring in coarser sediments<sup>11-13</sup> than *Capitella*, and having relatively small (190–230- $\mu$ m metamorphic length), planktotrophic larvae that live and feed in the plankton for weeks.

In separate experiments, we gave laboratory-reared larvae of

## PLASTIC SPHERES

## STILL WATER

|   |   |   |   |   |
|---|---|---|---|---|
| 3 | 6 | 4 | 3 | 2 |
| 3 | 5 | 4 | 5 | 4 |
| 5 | 6 | 4 | 7 | 9 |
| 7 | 7 | 8 | 4 | 2 |
| 8 | 6 | 5 | 6 | 3 |

## *Capitella* sp. I

|    |    |    |    |    |
|----|----|----|----|----|
| 71 | 1  | 57 | 4  | 49 |
| 2  | 62 | 1  | 72 | 0  |
| 60 | 0  | 53 | 0  | 43 |
| 5  | 68 | 1  | 43 | 0  |
| 69 | 0  | 55 | 1  | 57 |

## FLOW

|   |   |   |   |   |
|---|---|---|---|---|
| 1 | 0 | 3 | 3 | 3 |
| 2 | 0 | 0 | 4 | 0 |
| 0 | 3 | 0 | 1 | 1 |
| 2 | 0 | 1 | 1 | 4 |
| 0 | 3 | 0 | 1 | 0 |

|    |    |    |    |    |
|----|----|----|----|----|
| 11 | 0  | 24 | 0  | 25 |
| 4  | 21 | 1  | 29 | 0  |
| 7  | 0  | 10 | 0  | 13 |
| 1  | 10 | 1  | 14 | 0  |
| 13 | 0  | 11 | 0  | 22 |



Fig. 1 Patterns of *Capitella* sp. I larval settlement in still water (experiment 1) and in the flume (experiment 4). Refer to Table 1 for conditions during these experiments and to Table 2 for other results. Number of larvae and spheres per compartment are shown.

these two species a choice of two sediment treatments: a natural, organic-rich, muddy sediment (76.1%  $\leq 63 \mu$ m, frozen and thawed before use) and an abiotic, glass-bead (Ferro Class IVA 'Microspheres') mixture, with a similar grain-size distribution (70.0%  $\leq 63 \mu$ m). Experiments were conducted in a still-water box (50  $\times$  50  $\times$  30-cm deep) and in a racetrack-design, laboratory flume (6-m long straightaway, 50  $\times$  30-cm deep channel) where water was recirculated by a paddle wheel, producing a steady, unidirectional, smooth-turbulent flow. The box and flume were filled with 1- $\mu$ m filtered seawater to a depth of 10 cm. The shear velocity ( $u_*$ ) of the flow was 0.30 cm s<sup>-1</sup> ( $R^2 = 0.989$ ; estimated

Table 1 Conditions during habitat selection experiments

| Experiment number | Species           | Date     | Flow*<br>$x \pm 1$ s.d.<br>(cm s <sup>-1</sup> ) | Time of experiment       | Water temperature (°C) | Salinity (p.p.t.) | Sphere† diameter ( $\mu$ m) |
|-------------------|-------------------|----------|--------------------------------------------------|--------------------------|------------------------|-------------------|-----------------------------|
| 1                 | <i>Capitella</i>  | 2/09/87  | none                                             | 12:45–14:45 (2 h)        | 16.2                   | 31.5              | 200‡                        |
| 2                 | <i>Capitella</i>  | 4/22/87  | $4.8 \pm 0.2$ ( $N = 54$ )                       | 13:45–15:45 (2 h)        | 17.5                   | 31.5              | 200                         |
| 3                 | <i>Capitella</i>  | 6/24/87  | $5.3 \pm 0.2$ ( $N = 96$ )                       | 01:30–03:30 (2 h)§       | 20.5                   | 32.2              | 280                         |
| 4                 | <i>Capitella</i>  | 7/15/87  | $5.1 \pm 0.2$ ( $N = 102$ )                      | 10:30–12:30 (2 h)        | 22.8                   | 32.3              | 390                         |
| 5                 | <i>Mercenaria</i> | 4/08/87  | none                                             | 10:00–14:00 (4 h)        | 16.5                   | 31.5              | 390                         |
| 6                 | <i>Mercenaria</i> | 7/26/87  | $5.2 \pm 0.1$ ( $N = 102$ )                      | 10:40–14:40 (4 h)        | 23.2                   | 32.0              | 390                         |
| 7                 | <i>Mercenaria</i> | 12/20/87 | none                                             | 07:40–12:00 (4 h 20 min) | 15.6                   | 32.2              | 390                         |
| 8                 | <i>Mercenaria</i> | 12/20/87 | $5.0 \pm 0.2$ ( $N = 102$ )                      | 09:05–13:25 (4 h 20 min) | 14.5                   | 32.2              | 390                         |

\* Flow speed was monitored with a Marsh–McBirney electromagnetic current meter positioned in the middle of the channel, 1.5-m downstream from the leading edge of the sediment array, and 7.0-cm above the bottom. Speeds were recorded every 10 s for 1–2 wheel revolutions near the beginning and end of each experiment. Means for the two intervals agreed to within a few percent (and were often identical), so only means from the first measurement interval are reported here.

† Polystyrene DVB microspheres (Duke Scientific), with a particle density of 1.05 g cm<sup>-3</sup>. For *Capitella*, spheres initially corresponded to the mean fall velocities of anesthetized larvae, but larger, faster-falling spheres were used in later experiments, to account for downward swimming by the larvae.

‡ The spheres were not actually introduced into the still-water box during this particular *Capitella* experiment, but during a trial run for technique development, using an empty array.

§ This experiment was conducted completely in the dark—on a moonless night with all the room lights switched off.

**Table 2** Results of the habitat selection experiments

| Experiment number             |          | Number added | Number in array (%) | Number in mud $x \pm s.d.$ $N = 13$ | Number in beads $x \pm s.d.$ $N = 12$ | Number in water column* | Number in wash† | Total number (%) | Significance‡ |
|-------------------------------|----------|--------------|---------------------|-------------------------------------|---------------------------------------|-------------------------|-----------------|------------------|---------------|
| <i>Capitella</i> experiments  |          |              |                     |                                     |                                       |                         |                 |                  |               |
| 1                             | Larvae   | 1,200        | 774 (64.5)          | $58.4 \pm 9.9$                      | $1.2 \pm 1.7$                         | 10                      | 54              | 838 (69.8)       | **            |
|                               | Spheres§ | 200          | 127 (63.5)          | $4.9 \pm 2.0$                       | $5.2 \pm 1.9$                         | NC                      | NC              | —                |               |
| 2                             | Larvae   | 1,200        | 291 (24.2)          | $17.3 \pm 9.8$                      | $5.5 \pm 4.3$                         | 16                      | 66              | 373 (31.1)       | **            |
|                               | Spheres  | 1,200        | 35 (2.9)            | $1.4 \pm 2.1$                       | $1.4 \pm 1.1$                         | 17                      | 1,433           | 1,485 (123.8)    |               |
| 3                             | Larvae   | 1,200        | 221 (18.4)          | $16.7 \pm 14.3$                     | $0.3 \pm 0.5$                         | NC                      | NC              | —                | **            |
|                               | Spheres  | 1,200        | 13 (1.1)            | $0.5 \pm 1.1$                       | $0.5 \pm 0.7$                         | NC                      | NC              | —                |               |
| 4                             | Larvae   | 1,200        | 217 (18.1)          | $16.2 \pm 7.1$                      | $0.6 \pm 1.2$                         | NC                      | NC              | —                | **            |
|                               | Spheres  | 1,200        | 33 (2.8)            | $1.0 \pm 1.4$                       | $1.6 \pm 1.4$                         | NC                      | NC              | —                |               |
| <i>Mercenaria</i> experiments |          |              |                     |                                     |                                       |                         |                 |                  |               |
| 5                             | Larvae   | 3,000        | 657 (21.9)          | $3.7 \pm 2.3$                       | $50.8 \pm 19.6$                       | 623                     | 1,666           | 2,946 (98.2)     | **            |
|                               | Spheres  | 2,000        | 621 (31.0)          | $21.6 \pm 6.1$                      | $28.3 \pm 7.5$                        | 100                     | 1,132           | 1,853 (92.7)     | *             |
| 6                             | Larvae   | 3,000        | 41 (1.4)            | $1.2 \pm 1.1$                       | $2.2 \pm 1.3$                         | 1,680                   | 880             | 2,601 (86.7)     |               |
|                               | Spheres  | 2,000        | 58 (2.9)            | $2.5 \pm 1.5$                       | $2.2 \pm 1.3$                         | 0                       | 1,470           | 1,528 (76.4)     |               |
| 7                             | Larvae   | 3,000        | 364 (12.1)          | $2.9 \pm 2.0$                       | $27.3 \pm 6.8$                        | 38                      | 798             | 1,200 (40.0)     | **            |
|                               | Spheres  | 3,000        | 837 (27.9)          | $32.3 \pm 12.4$                     | $34.8 \pm 14.1$                       | 3                       | 1,883           | 2,723 (90.8)     |               |
| 8                             | Larvae   | 3,000        | 35 (1.2)            | $0.9 \pm 1.1$                       | $2.0 \pm 1.4$                         | 12                      | 886             | 933 (31.1)       | *             |
|                               | Spheres  | 3,000        | 175 (5.8)           | $5.6 \pm 3.8$                       | $8.5 \pm 5.4$                         | 4                       | 1,660           | 1,839 (61.3)     |               |

NC = not counted.

\* At the end of each experiment, the lid was placed on the sediment array and the water down to within ~1 cm of the lid was drained through a 63- $\mu$ m sieve. The organisms and spheres enumerated from this fraction are reported as having been in the 'water column', although this is probably an underestimate in the flume, as the spheres and larvae could have fallen to within 1 cm of the bed during the 30-min draining period.

† After most of the water was drained from the still-water box or the flume (see footnote\*), the remaining water (including water that was below the array) and washings from all surfaces were passed through a 63- $\mu$ m sieve. The organisms and spheres enumerated from this fraction are reported as in the 'wash'.

‡ Probabilities (two-tailed) for rejecting the null hypothesis of no difference in collections between the mud and the glass bead treatments, using a Mann-Whitney *U* test: \*\* =  $p < 0.01$ ; \* =  $p < 0.05$ .

§ See footnote † to Table 1.

using the 'profile technique'<sup>14</sup>, from flow measurements obtained with a laser-Doppler velocimeter) indicating relatively low shear and turbulent mixing compared, for example, to  $u_* \leq 0.6 \text{ cm s}^{-1}$  estimated for the everyday tidal flows<sup>15</sup> and  $u_* > 3.0 \text{ cm s}^{-1}$  during storms (unpublished data; W. D. Grant, deceased 7 October 1986) in habitats common to both species.

The treatments were arranged in a checkerboard design in a five-by-five array of  $4 \times 4 \times 1$ -cm deep compartments (separated by 0.3-cm partitions) milled out of the central region of a 50-cm square section of the flume false bottom. Polystyrene spheres, with fall velocities similar to the mean fall velocities of the anesthetized larvae of each species, were also added during the experiments to determine passive fall-out onto the sediments. During or before each selection experiment, subsamples from the larval cultures were tested for competency to settle and metamorphose, to establish similar competency among larval broods for between-experiment comparison of substrate selection.

In still water, *Capitella* larvae clearly preferred the natural mud over the glass-bead treatment (Fig. 1, Table 2), but the passive spheres were randomly distributed between the two treatments. Larval preference for mud is consistent with the organism's natural field distribution<sup>7,8</sup>, and with the food requirements of the deposit-feeding adults. In contrast, *Mercenaria* larvae clearly chose glass beads over natural mud in still water (Fig. 2, Table 2). The preference of the larvae is again consistent with the food requirements of the filter-feeding adults in that relatively low-organic sediments (like the glass beads) may be associated with relatively erosional sediment-transport environments—areas of high advective flux of suspended material. This hypothesis is supported by results of other still-water experiments with *Mercenaria* where the choices included a natural sand (1.2% silt plus clay) treatment, as well as natural mud and glass beads. The larvae preferred sand over mud and beads over sand (C.M.W., C.A.B. and J.P.G., manuscript in

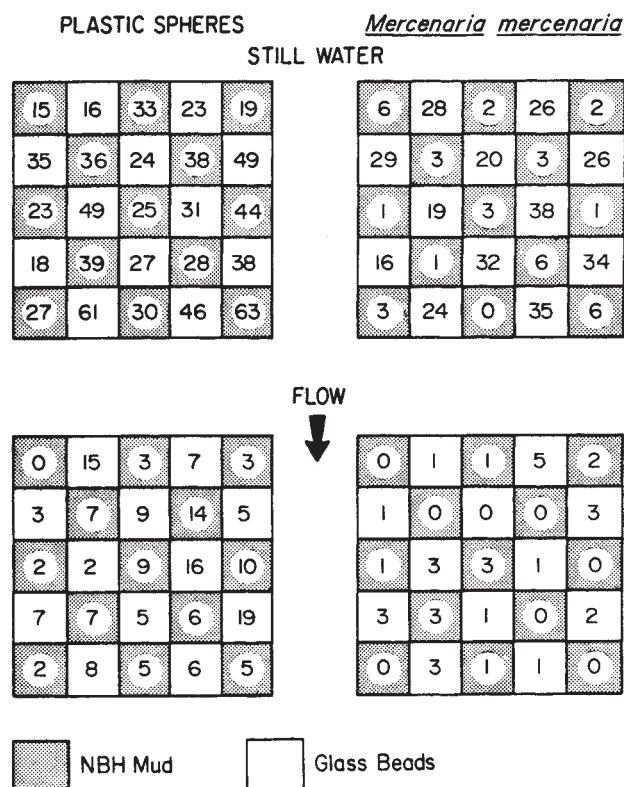
preparation) suggesting that they may be responding to low organic content, rather than to grain size.

In the flume experiments, *Capitella* larvae were significantly more abundant in the mud than in the beads (Fig. 1, Table 2), as in still water. The *Capitella* settlement response was very consistent; the mean number collected in the mud treatment was similar for three flow experiments (Table 2), which varied in temperature, light regime (variables that could not be controlled in this large flume), and larval brood (Table 1). In contrast to the *Capitella* flume results and the *Mercenaria* still-water results, mean collections of *Mercenaria* larvae were not significantly different between the two sediment treatments in both flume experiments (Fig. 2, Table 2). Also in contrast to the *Capitella* experiments, similar percentages of *Mercenaria* larvae and spheres were collected in the flume array, indicating that the clams were deposited more like passive particles than were the worms (Table 2).

The difference in total *Mercenaria* settlement between still water and the flume flow cannot be explained by differences in larval competency because the same larval brood was used for still-water experiment 7 and flow experiment 8, which were conducted within 2 h of each other (see Table 1). Although *Mercenaria* exhibited relatively low settlement (compared with *Capitella*) and showed no sediment preference in the flume flow tested here, enhanced settlement and substrate selection by this species may occur under slow flow conditions or over a period longer than the 4-h interval tested. But,  $u_* \geq 0.3 \text{ cm s}^{-1}$  occurs during  $\leq 3$ -h intervals over the tidal cycle in Buzzards Bay, Massachusetts (USA)<sup>15</sup>, where both species are common, so the 4-h interval tested is realistic for *Mercenaria*'s natural habitat and for similar coastal embayments.

Between-species differences in settlement patterns in the flume flow, compared to still water, may be related to differences in the swimming and search behaviours of the competent larvae. Video observations in still water<sup>16</sup> indicate that competent worm





**Fig. 2** Patterns of *Mercenaria mercenaria* larval settlement in still water (experiment 7) and in the flume (experiment 8). Refer to Table 1 for conditions during these experiments and to Table 2 for other results. Number of larvae and spheres per compartment is shown.

larvae move primarily in the horizontal plane and remain within a centimeter of the bed, frequently swimming down to the bottom, evidently to test the substrate, but swimming away in the absence of an appropriate cue<sup>9,10</sup>. In contrast, swimming by clam pediveligers involves a large proportion of vertical spirals, in addition to horizontal circles, and directed horizontal swimming is less common. Due to advection and mixing processes, this vertical swimming behaviour may render *Mercenaria* larvae more unavailable for settlement onto the bed than the worm larvae, which stay very close to the bottom and frequently swim down to test the substrate. Furthermore, *Mercenaria* postlarvae attach to the substrate by a temporary byssal thread which they frequently release, in contrast to other clam species living in similar habitats which attach with a strong byssus network upon settlement<sup>17</sup>. Thus, the weak attachment of settled *Mercenaria* may facilitate dislodgement and resuspension in the flow tested here.

These are the first larval settlement experiments conducted under controlled and defined laboratory flow conditions, and this is the first demonstration that an infaunal species (*Capitella* sp. I) can actively select a preferred habitat in a realistic, turbulent flow. Furthermore, the different flume results for *Capitella* and *Mercenaria* indicate that selection experiments conducted in still water only are not sufficient to infer habitat selection in natural field flows. Extending this experimental approach to a wide range of flow regimes, sediment treatments, spatial scales, and larval species is now required to determine the capabilities and the conditions under which these choices can be effected. This information is needed to evaluate the relative importance of hydrodynamics and larval behaviours in explaining patterns of larval settlement and, ultimately, in determining the role of settlement processes in the development and maintenance of infaunal species distributions.

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## Neural encoding of individual words and faces by the human hippocampus and amygdala

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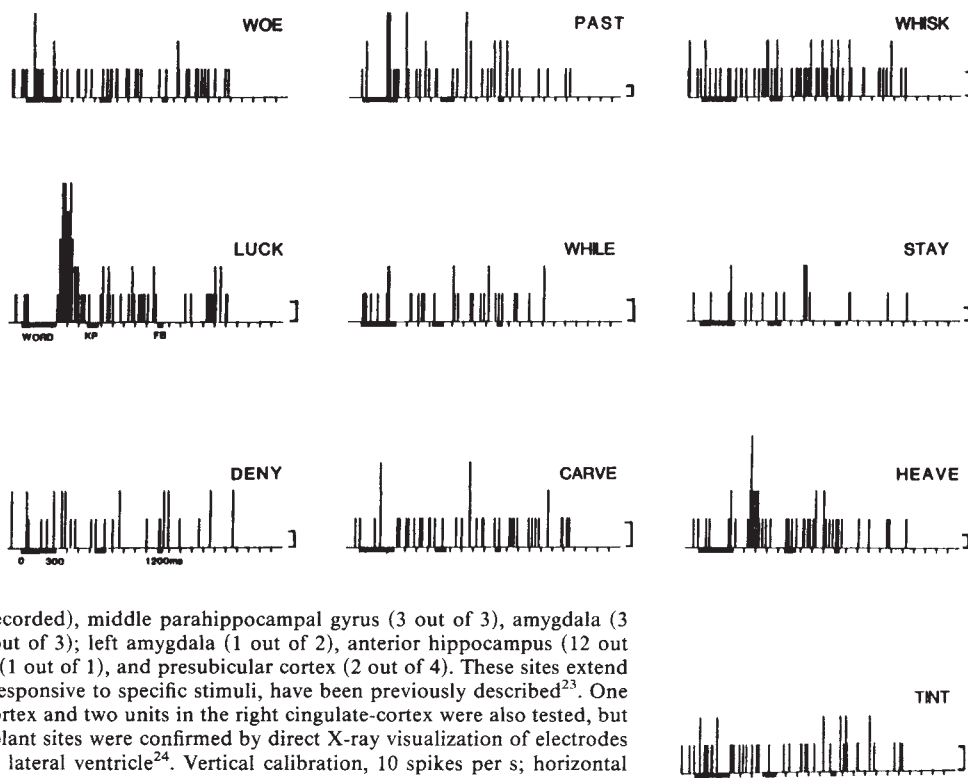
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Patients with lesions in the medial temporal lobe (MTL) of the brain, which includes the hippocampus, amygdala and parahippocampal gyrus, are severely impaired in their ability to remember and recognize words or faces which they saw only a short time ago<sup>1,2</sup>. These lesions also prevent the effect of word repetition on cortical event-related potentials that are associated with these tasks<sup>3</sup>. We have been able to study the response of individual neurons in the human medial temporal lobe to such delayed recognition tasks in epileptic patients undergoing neurosurgery. We found that some MTL neurons preferentially fired on sight of one particular word from a set of ten words used in a memory task, and others fired in response to one particular face. This stimulus-specific firing was maximal during the time that the neocortical event potentials are most sensitive to stimulus repetition, suggesting that the MTL contributes specific information to the cortex during the retrieval of recent memories<sup>4,5</sup>.

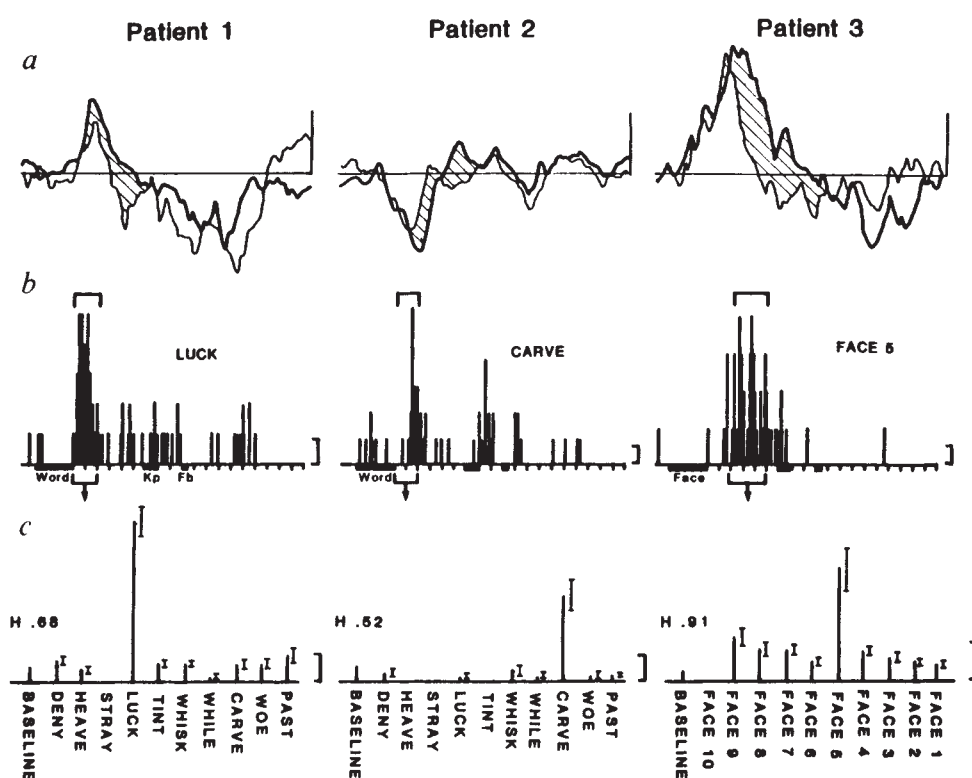
Complex partial epilepsy that is resistant to medication can be treated successfully by surgical resection after the epileptogenic tissues have been identified with depth electrodes<sup>6</sup>. Based on clinical criteria, and after independently reviewed informed consent, bilateral MTL depth electrodes were implanted in ten patients. Action potentials were recorded from immobile insulated 40 µm wires<sup>7</sup> while common abstract words were presented on a videomonitor for 0.3 s every 2.5 s. The subject was instructed to memorize each of 20 words presented.

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**Fig. 1** Stimulus-specific excitation in the left anterior hippocampus. Each histogram is the unit's response to the nine presentations of that particular repeated word. The response to 'LUCK' was significantly greater than that to the other nine words ( $P < 0.0001$ ). The same unit is displayed in the left-most column of Fig. 2. Thirty-nine units in ten patients met the minimum requirements of firing rate ( $>0.5$  spike  $s^{-1}$ ), freedom from artifacts (epileptiform or movement), and good performance. All unit recordings were from sites distant or contralateral to the seizure foci. Because action potentials (signal:noise  $>2:1$ ) from different neurons sometimes overlap<sup>22</sup> and the recordings were from fixed electrodes, the 'units' in this study consist predominantly of the activity of one neuron, with an occasional spurious action potential from other cells. These units were located in the right anterior hippocampus (7 responsive out of 8 recorded), middle parahippocampal gyrus (3 out of 3), amygdala (3 out of 4), and presubicular cortex (1 out of 3); left amygdala (1 out of 2), anterior hippocampus (12 out of 14), middle parahippocampal gyrus (1 out of 1), and presubicular cortex (2 out of 4). These sites extend the locations where primate neurons, responsive to specific stimuli, have been previously described<sup>23</sup>. One unit in the left supplementary-motor-cortex and two units in the right cingulate-cortex were also tested, but lacked stimulus-specific responses. Implant sites were confirmed by direct X-ray visualization of electrodes and of the hippocampus indenting the lateral ventricle<sup>24</sup>. Vertical calibration, 10 spikes per s; horizontal scale, 100 ms per division.



**Fig. 2** Responses of hippocampal neurons to a particular word or face in three patients. Displayed are simultaneous averaged field potentials (*a*), sum histograms of neuronal firing in response to preferred stimuli (*b*), and total number of action potentials in response to all repeated stimuli during a selected time period (*c*), for three left anterior hippocampal units. *a*, Stimulus repetition produces a smaller, earlier N4 (nonrepeated versus repeated N4/P3 difference is shaded). The e.r.p.s are averages of all repeated (thin lines) and nonrepeated (thick lines) stimuli. Each e.r.p. was recorded from the same electrode as the accompanying unit data. The N4 in column 2 is positive presumably because it was recorded across the N4 reversal plane, near the N4 generating synapses<sup>8,13</sup>. Vertical calibration, 50  $\mu V$ . Negative is up. *b*, Time course of the stimulus-specific responses to the nine presentations of the preferred word (patient 1, 2) or face (patient 3). Vertical calibration, 10 spikes per s; horizontal scale, 100 ms per division. *c*, The unit response to the specific stimulus ( $P < 0.0001$ ) is compared to that of the other nine repeated stimuli ( $P > 0.1$ ). Displayed is the total activity ( $\pm$ s.e.) for the time window bracketed in (*b*). Vertical calibration, 5 spikes per s. The activity in response to all nine stimulus presentations was summed for the time windows bracketed in (*b*). These windows were one of four used for statistical analysis. The statistic was applied to four time-windows defined by the hippocampal cognitive-field potentials N4 and P3<sup>8</sup>. The four windows were: preceding the N4 (230–400 ms), during the N4 (300–500 ms) or P3 (470–770 ms), or following the P3 (800–1,200 ms). For a given time-window and unit, the total number of action potentials was summed for all nine presentations of the stimulus. This number was then compared with the population distribution (estimated from 10,000 samples) obtained by summing nine random samples of activity evoked by any of the ten stimuli. All *p* values cited were confirmed with and without replacement of missing values, which were lost due to artifact rejection<sup>25</sup>. Abbreviations: Kp, averaged keypress; Fb, feedback tone; H, breadth of tuning index<sup>9,10</sup>.





Following a brief pause, eight sequential recognition tests occurred with no interruptions. The same 10 words from the initial 20 were repeated in each testing block, intermixed with ten new words (nonrepeats) that only occurred once in all eight blocks. Thus, each repeated word occurred a total of nine times whereas the other words only occurred once. The subjects were instructed to press a key when they saw repeated words<sup>8</sup>. Out of 39 recorded units tested among the 10 subjects, 30 gave visually and statistically significant evidence ( $P < 0.005$ ) for specific activation in response to one of the ten repeated words (Fig. 1). Fourteen of these units were tested in a similar way with black and white photographs of nondescript white male faces. Two left anterior hippocampal units, both responsive to a particular word, also responded preferentially to one of the repeating faces (Fig. 2). All 30 responsive units showed increased firing in response to their preferred stimulus beginning 300–500 ms after stimulus onset and lasting for 200–500 ms. The mean breadth-of-tuning index<sup>9,10</sup> was 0.77 (s.d. = 0.21).

In contrast to the stimulus-specific response, visual sensory responses in the human MTL are concentrated in the posterior hippocampal formation and have latencies less than 100 ms<sup>11</sup>. Of the 39 units described here, 31 were given a visual sensory input, a reversing checkerboard pattern; none responded.

Two variants of the recognition task were used to examine the effects of pressing a key on the stimulus-specific responses. In one, six of the patients (30 units) were tested with an additional five blocks of the same word set, during which they were instructed to 'just watch and not respond'. In the second, one of these patients, plus four additional patients (14 units), were tested with a new set of words, but asked to press the key in response to nonrepeating words. Six units had stimulus-specificity in the 'just watch' tests, while eight units developed specific responses to a repeating word even when not a keypress target. Furthermore, stimulus-specific neuronal responses usually began 300–500 ms before the overt behavioural responses (namely, pressing the key). Thus, the stimulus-specific responses were not directly related to the key-pressing.

The specific firing is too common, given our limited sampling and the large number of words in the average vocabulary, to avoid the conclusion that these cells would fire in response to other words in other circumstances. In fact, of the 13 units that responded to a particular word, and were then tested with a second set of words, 8 responded specifically to a different word in the second set. Furthermore, 13 of the 30 units that responded specifically to one word also responded less strongly, but still at a statistically significant level, to another word in the same stimulus set. These data suggest that MTL stimulus-specific responses represent a temporary allocation of a subset of MTL neurons to the ensemble encoding of distinct events within a given context. By the same reasoning, it is likely that had we sampled more neurons, we would have found other cells simultaneously responding to the same word.

The effects of transient electrical disruption of MTL physiology suggest that the MTL makes its essential contribution to recognition in the second after stimulus presentation<sup>12</sup>. During this time, summed synaptic activity in both the MTL and the neocortex generates two event-related potentials (e.r.p.s): the N4 followed by the P3<sup>8,13</sup>. The timing and the behavioural correlates of the N4 are consistent with it reflecting neural encoding of the stimulus within its cognitive context<sup>4</sup>. The N4 begins about 300 ms after a word is presented and repetition of a word causes the event-related potential to decrease in response to repeated compared to new (nonrepeated) words<sup>8</sup>. This repetition-induced N4 decrease depends critically upon structures in the left temporal lobe<sup>3</sup>. Stimulus-specific neuronal responses begin concurrently with such N4 attenuation (Fig. 2), suggesting that they may contribute to the repetition-induced reduction of the N4 by feeding back to the association cortex at the onset of stimulus encoding, and thus permitting the encoding to proceed more rapidly in response to repeated stimuli.

Repetition-induced changes in overall neuronal firing have been found in the anterior thalamus and inferotemporal lobe of primates<sup>14,15</sup>. In contrast, the stimulus-specific units studied here showed no change in overall firing rate in response to repeated versus nonrepeated stimuli. The specific response of a unit to its preferred stimulus represents a selective distribution of its activity in response to that stimulus, rather than a general increase in firing in response to all repeated stimuli in the set. Furthermore, as that particular stimulus was repeatedly presented, there was in general no increase in firing from that evoked by its initial presentation. Thus, the contribution of the MTL to recognition does not appear to result from a gross increase in its output when a stimulus is repeated.

This is in apparent conflict with theories of hippocampal function which suggest that long-term potentiation (LTP), a proposed mechanism for memory trace formation<sup>16</sup>, results in increased hippocampal discharge in response to repeated stimuli. The role of LTP may in fact be in the stabilization of stimulus specificity in hippocampal cell firing. In this view, the strengthening of particular synapses by LTP at the time of the initial stimulus presentation would promote the reactivation of the same cells when the same stimulus was re-presented. Feed-forward and feedback inhibition would then prevent the increased synaptic specificity from generating an increase in firing on re-presentation<sup>3,4,17</sup>.

Current theories postulate that the elements of an episodic cognitive memory are linked as a weak network of association-cortex neurons. Some models suggest that the hippocampus promotes the initial formation, subsequent retrieval, and/or the consolidation of this network through nonspecific modulatory effects<sup>18,19</sup>. Other models<sup>5,20,21</sup> suggest that the MTL promotes selective recruitment of the particular neurons constituting the neocortical representation of a memory. Overall, the data reported here suggest that the MTL contributes specific information rather than diffuse modulation to the encoding and subsequent recognition of a stimulus during recent memory.

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# HTLV-1 transactivator induces interleukin-2 receptor expression through an NF- $\kappa$ B-like factor

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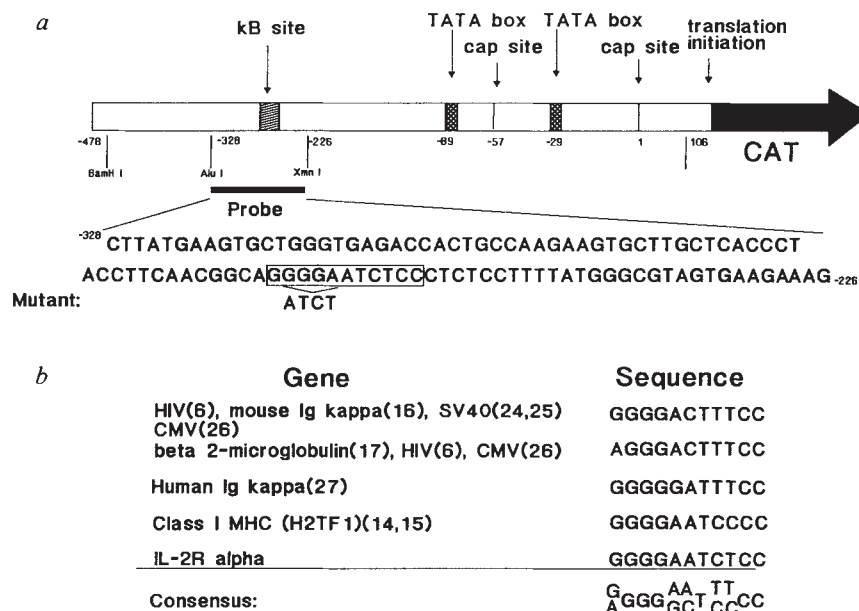
Like other viruses that infect primate cells, the human T lymphotropic virus-I (HTLV-I) stimulates production of some host cell proteins. In particular, HTLV-I infected T cells synthesize interleukin-2 receptor  $\alpha$  (IL-2R $\alpha$ ) chain, which is probably induced through the mediation of the *tat*-I gene product of the virus<sup>1-5</sup>. Activated T cells contain a transcription factor called NF- $\kappa$ B<sup>6</sup>, which stimulates the expression of human immunodeficiency virus-1 (HIV-1) by binding to an 11-base-pair enhancer sequence called  $\kappa$ B. We have now found evidence that a similar transcription factor is involved in the induction of IL-2R $\alpha$  expression by *tat*-I. We have identified a sequence upstream of IL-2R $\alpha$  which is the same as the  $\kappa$ B site at 9 of 11 base pairs, competes for binding to the  $\kappa$ B sequence, and serves as a *tat*-I responsive element when multiple copies are inserted upstream of a heterologous promoter. The *tat*-I product also induces  $\kappa$ B and the IL-2R $\alpha$   $\kappa$ B binding activity in transfected Jurkat T lymphoid leukaemia cells. Both HTLV-I and HIV-1 thus interact with NF- $\kappa$ B-like transcription factors which might normally regulate expression of a growth factor receptor gene.

Stimulation of T cells leads to the expression of NF- $\kappa$ B, which recognizes an 11-base-pair (bp) DNA sequence twice repeated in the HIV enhancer<sup>6</sup>; however, normal T-cell specific targets

of NF- $\kappa$ B were unknown. Because the upstream regulatory region of IL-2R $\alpha$  contains a site similar to  $\kappa$ B (Fig. 1b), we analysed its potential role in IL-2R $\alpha$  activation. To determine whether the IL-2R $\alpha$  site was recognized by NF- $\kappa$ B, we performed electrophoretic mobility shift assays using a  $\kappa$ B probe. Double-stranded oligonucleotide fragments containing the IL-2R $\alpha$   $\kappa$ B site (Fig. 2a, lanes 4, 5) and the immunoglobulin  $\kappa$ B site (Fig. 2a, lanes 2, 3) competed equally and specifically for binding, in contrast to an unrelated IL-2 site. Using a radiolabelled probe containing the IL-2R $\alpha$   $\kappa$ B sequence, an inducible complex was identified (Fig. 2b, lane 2) which also competed with the HIV  $\kappa$ B site (Fig. 2b, lanes 3, 4) or the IL-2R $\alpha$   $\kappa$ B site (Fig. 2b, lanes 5, 6) fragments. Binding to the  $\kappa$ B-like site in the native IL-2R $\alpha$  enhancer was confirmed using a probe from the upstream region (Fig. 2c, lane 2). HIV or IL-2R $\alpha$   $\kappa$ B fragments competed with the  $\kappa$ B-like site for formation of the inducible complex (data not shown). When a similar probe modified at four base pairs in the IL-2R $\alpha$   $\kappa$ B site (Fig. 1a) was used, this specific inducible complex did not form (Fig. 2c, lane 3). Finally, transfection of *tat*-I into Jurkat cells resulted in induction of both  $\kappa$ B- (Fig. 2d, lane 2) and IL-2R $\alpha$   $\kappa$ B- (Fig. 2d, lane 4) binding proteins.

The role of this  $\kappa$ B-like site in IL-2R $\alpha$  gene expression was determined with a bacterial plasmid containing the upstream region of IL-2R $\alpha$  (-479 to +106) linked to the CAT gene (Fig. 1a). Expression of this plasmid was compared to the mutant plasmid by transient transfection. CAT activation in analogous plasmids has been shown previously to correlate with increased messenger RNA levels<sup>4,7</sup>. CAT activity in cells incubated with 12-O-tetradecanoyl phorbol 13-acetate (TPA) was induced by four- to sixfold (Fig. 3a). Comparable stimulation was seen in the mutant plasmid, suggesting that the IL-2R $\alpha$   $\kappa$ B site is not responsive to TPA.

The mechanism of *tat*-I activation was examined by cotransfection of IL-2R $\alpha$ -CAT with a plasmid that could express *tat*-I protein. IL-2R $\alpha$ -CAT activity increased sixfold in the presence of the *tat*-I plasmid; however, no *tat*-I stimulation of the mutant plasmid was observed (Fig. 3b). The function of the



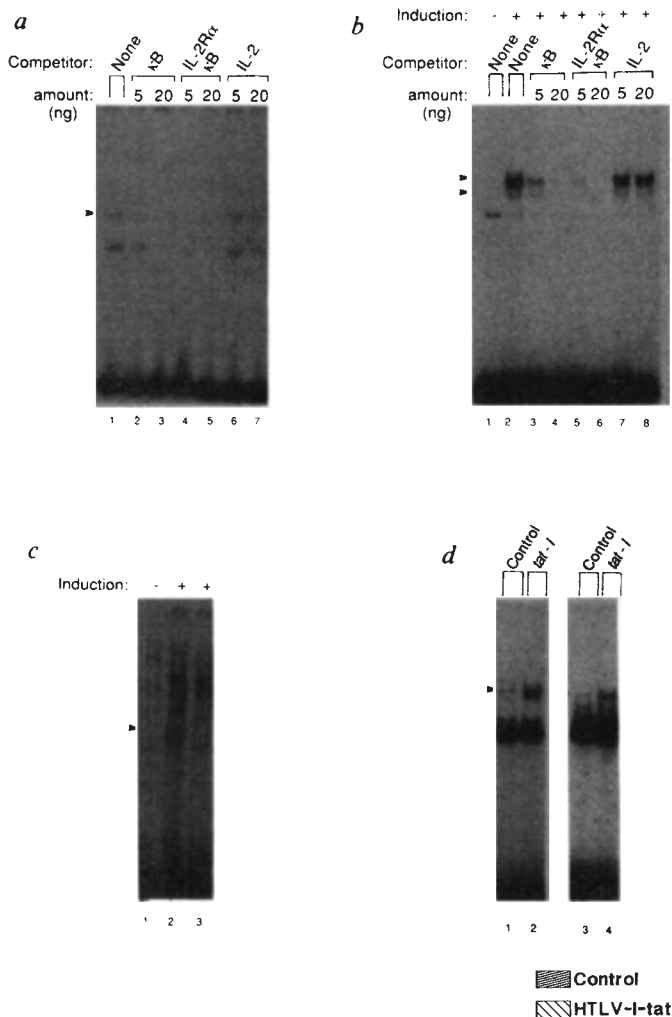
**Fig. 1** **a**, Schematic representation of the IL-2 receptor  $\alpha$  chain upstream sequence. Four cap sites and associated TATA boxes have been defined, but only two are major transcriptional start sites; TATA boxes associated with these are indicated. The most downstream cap site is identified as +1. The probe from IL-2R $\alpha$  used for binding gel analysis is shown. Bases altered in the mutant plasmid are indicated below the wild type sequence. **b**, Sequences of  $\kappa$ B-related sites and their associated genes.

**Methods.** A plasmid containing a 1,352-bp *Pst*I fragment of IL-2R $\alpha$  upstream region<sup>3</sup>, kindly provided by Dr J. Depper, was digested with *Eco*RI and *Pst*I, incubated with T4 polymerase, and a 585-bp insert was isolated. This insert was ligated to p106-CAT<sup>18</sup>, provided by Dr M. Gilman, previously digested with *Sma*I and calf intestinal phosphatase (CIP), and correct orientation determined by digestion with *Bam*HI. The mutant plasmid with the indicated base pair changes was derived by site-directed mutagenesis as previously described<sup>6</sup>.



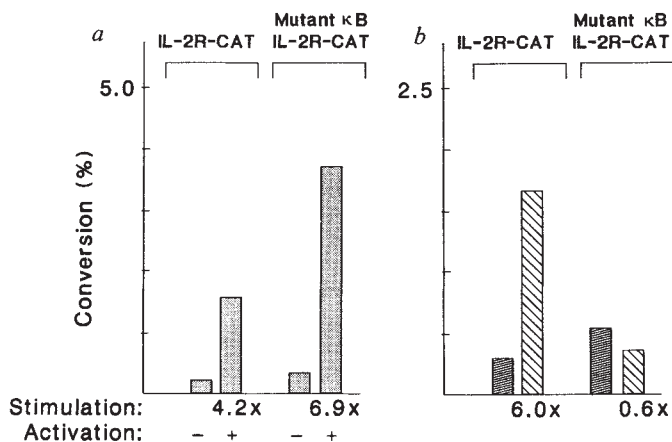
**Fig. 2** Induction and specificity of factor binding to the  $\kappa$ B-like site in Jurkat cells by phorbol esters and *tat*-I. Analysis of binding-site specificity and competition studies using electrophoretic mobility-shift assay with radiolabelled probe containing *a*,  $\kappa$ B sites from HIV enhancer; *b*, IL-2R $\alpha$   $\kappa$ B sites (double-stranded oligonucleotide probe); *c*, IL-2R $\alpha$  probe, wild type (lanes 1, 2) or mutant (lane 3; fig. 1*a*). Nuclear extracts (8  $\mu$ g) from unstimulated (–) or induced (+) Jurkat cells (40 nM TPA treatment for 4 h at 37 °C) were incubated with the relevant radiolabelled DNA probe alone or in the presence of indicated amounts of unlabelled competitors, double-stranded oligonucleotide containing a  $\kappa$ B site, IL-2R $\alpha$   $\kappa$ B site or a control (unrelated) fragment of the same size from the IL-2 promoter (B site)<sup>19</sup>. *d*, Extracts (25  $\mu$ g) prepared from Jurkat cells transfected with HTLV-I-*tat* or pGEM-2, which resembles the vector containing the *tat*-I gene, were incubated with radiolabelled double-stranded oligonucleotide probe from  $\kappa$ B sites of the HIV enhancer (lanes 1, 2) or IL-2R $\alpha$  (lanes 3, 4) as above. Binding of the specific inducible complexes (see arrows) using the IL-2R $\alpha$  probe (*c*),  $\kappa$ B or IL-2R $\alpha$   $\kappa$ B (*d*) also competed specifically with both  $\kappa$ B-like sites. All samples contained poly dIdC (1  $\mu$ g).

**Methods.** Nuclear extracts were prepared according to the method of Dignam *et al.*<sup>20</sup>, and the electrophoretic mobility shift assay performed as previously described<sup>6</sup>. Radiolabelled  $\kappa$ B site probe from HIV enhancer was prepared from a 93-bp *Hae*III fragment from the HIV enhancer<sup>6</sup>. A double-stranded oligonucleotide probe for the IL-2R $\alpha$   $\kappa$ B site was derived from sequence –267 to –252 of the IL-2R $\alpha$  receptor upstream region (CAGGGGAATCTCCCTC). The double-stranded oligonucleotide probe for the  $\kappa$ B site was used as competitor and as radiolabelled probe in *d*. Its sequence was derived from the HIV enhancer, GATCAGGGACTTTCCGCTGGGGACTTTC. IL-2R $\alpha$  probe (Fig. 1) was prepared by isolation of a *Bam*HI to *Xmn*I fragment from IL-2R $\alpha$ -CAT, digestion with *Alu*I, treatment with CIP, phenol and chloroform extraction, ethanol precipitation, and T4 polynucleotide kinase labelling. A mutant IL-2R $\alpha$  probe was prepared as above using the mutant  $\kappa$ B site IL-2R $\alpha$ -CAT plasmid. HTLV-*tat*-I plasmid<sup>21</sup> was kindly provided by Dr Kuan-Teh Jeang. Jurkat cells (10<sup>7</sup>) were transfected with the relevant HTLV-I-*tat* or control plasmid (20  $\mu$ g) using DEAE-dextran<sup>6</sup>, and nuclear extracts were prepared after 48 h.



**Fig. 3** Mutant IL-2R $\alpha$ -CAT expression is stimulated by phorbol esters but not by the *tat*-I gene product. *a*, IL-2R $\alpha$ -CAT or mutant IL-2R $\alpha$ -CAT plasmid (20  $\mu$ g) was transfected into Jurkat cells (10<sup>7</sup>) using DEAE-dextran. After 16–20 h, cells were left untreated (–) or incubated with 40 nM TPA (+) (Sigma, St Louis, Mo) for the last 20 h of culture before harvest at 44 h. *b*, IL-2R $\alpha$ -CAT or mutant plasmid (20  $\mu$ g) was cotransfected as above with a HTLV-*tat*-I or a control (HTLV-I-*tat* deletion mutant) plasmid (5  $\mu$ g). CAT activity was determined 44 h later.

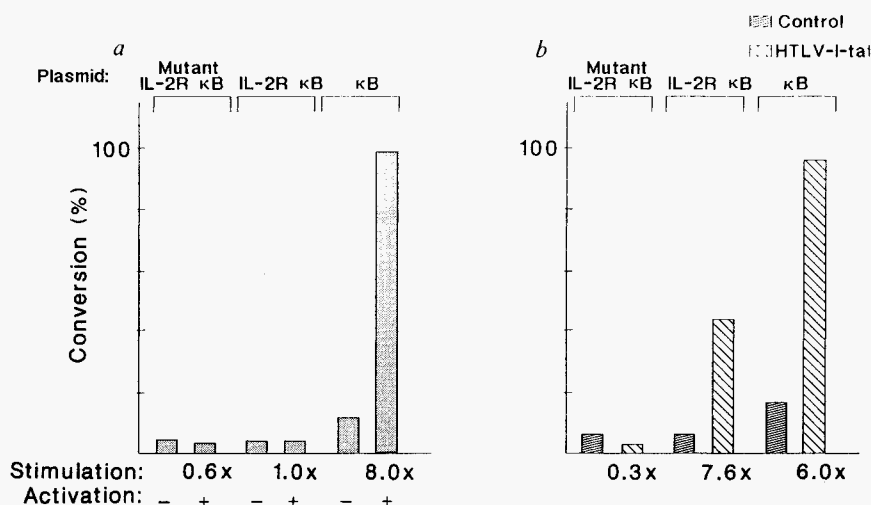
**Methods.** Cells (10<sup>7</sup>) were transfected and maintained as previously described<sup>6</sup>. The HTLV-*tat*-I deletion mutant control was prepared by digestion with *Acl*I and *Cla*I, isolation of linear fragment, and ligation with T4 DNA ligase, removing a 126-bp fragment near the 5' end of the *tat*-I coding sequence. Cell extracts were prepared, protein concentration assayed, and transfection efficiencies standardized as described<sup>1</sup>, or using cotransfection with an expression plasmid (5  $\mu$ g) containing an IL-3 cDNA, measuring IL-3 activity as previously described<sup>22</sup>. CAT activity was determined according to standard methods<sup>6,23</sup>. Degree of conversion was determined by removing spots containing either unreacted <sup>14</sup>C-chloramphenicol or acetylated forms and measuring the amount of radioactivity in a liquid scintillation counter. Mutant plasmid was derived by site-directed mutagenesis as described in Fig. 1. Standard deviations for each CAT assay were less than 10%, and results are representative of at least six independent transfections.



IL-2R $\alpha$   $\kappa$ B site in the absence of the other potential IL-2R $\alpha$  regulatory sites was analysed using plasmids containing four copies of the different  $\kappa$ B sites linked to the simian virus 40 (SV40) promoter and CAT gene. Whereas both *tat*-I and TPA stimulated CAT activity six- or eightfold in the  $\kappa$ B plasmid, only *tat*-I activated expression of IL-2R $\alpha$   $\kappa$ B site plasmid (Fig. 4). Because these plasmids are otherwise identical except for two base pairs in the enhancer, one or both of these bases probably confers TPA inducibility. No stimulation by *tat*-I or

TPA was seen in a plasmid with a mutation in the IL-2R $\alpha$   $\kappa$ B sites, showing that *tat*-I stimulation is dependent on recognition of the IL-2R $\alpha$   $\kappa$ B site (Fig. 4b).

Our analysis shows that the IL-2R $\alpha$   $\kappa$ B site is required for *tat*-I induction but not for TPA stimulation. The mechanism of stimulation by *tat*-I is unknown, but it probably induces an NF- $\kappa$ B-like factor rather than recognizing the site directly. There is little evidence that *tat*-I protein binds to a specific sequence of DNA. In fact, *tat*-I may activate more than one transcription factor



**Fig. 4** Stimulation of plasmid containing IL-2Rα κB sites linked to a heterologous promoter by phorbol esters and the *tat-I* gene product. **a**, Jurkat cells were transfected with modified pSP-CAT plasmids, provided by Dr R. Sen, which contains the SV40 promoter linked to CAT. The plasmid was modified by inserting four copies of a mutant IL-2Rα κB, IL-2Rα κB, or κB site, upstream of the SV40 promoter. Cells were untreated (-) or activated with 40 nM TPA (+) as in Fig. 3. **b**, Mutant IL-2Rα κB, IL-2Rα κB or κB plasmids (20 μg) (see above) were transfected with the HTLV-*tat-I* plasmid (5 μg) or control plasmid (HTLV-*tat-I* deletion mutant) (5 μg) and maintained at 37 °C for 44 h, after which CAT activity was determined.

**Methods.** pSP-CAT was prepared by the isolation of *Bgl*II-*Xba*I fragment of pA10CAT2 containing the SV40 promoter linked to CAT, and ligation to a plasmid backbone prepared from SP-64 digested with *Bam*HI and *Xba*I. Four-copy insert plasmids were prepared by ligation of the following synthetic double-stranded oligonucleotide fragment to pSP-CAT digested with *Sac*I, *Sma*I and *CIP*: mutant IL-2Rα κB, GCTCAATCTCCAGAGCTCAATCTCCTCGAGCTCAATCTCCACTGCTCAATCTCTCTCGA; IL-2Rα κB, GGGGAATCTCCAGAGG-GGAATCTCTCTCGAGGGGAATCTCCACTGGGGAATCTCTCTCGA; κB, GGGGAATCTCCAGAGGGGACTTTCTCTCGAGGGGACTTTT-CCACTGGGGAATCTCTCTCGA. The anti-sense strands of each were complementary with an overhanging AGCT at the 3' end, creating a *Sac*I-compatible end. Maxam-Gilbert sequencing confirmed the presence of the relevant insert in each plasmid. Transfection, normalization and determination of CAT activity are detailed in Fig. 3.

because its target site in the HTLV-I LTR<sup>8-10</sup> is unrelated to the IL-2Rα κB site.

The *tat-I* product activates some viral enhancers containing κB such as SV40<sup>11</sup> but not others, for example the complete HIV enhancer<sup>12</sup>. It is not clear whether this specificity results from the interaction of a single gene product with κB-like sites, or many. Singh *et al.* have reported the isolation of a complementary DNA encoding a polypeptide which recognizes two different κB-like sequences<sup>13</sup>, the H2TF1 site of class I MHC genes<sup>14,15</sup> and immunoglobulin κB sequences<sup>6,16</sup>. The existence of this single copy gene, however, does not exclude the possibility that other proteins may recognize these sites. Our inducible complex co-migrates with NF-κB and competes in equimolar amounts for binding to the HIV κB sequence, but, unlike κB, the IL-2Rα κB-like site does not respond to TPA, raising the possibility of multiple κB recognition proteins. Alternatively, transcriptional activity of a single protein could be regulated by subtle differences in DNA sequence.

Analysis of cellular genes reveals that κB-like sites are associated with cell surface molecules (refs 14-17, Fig. 1b). It is possible that the NF-κB system evolved to control the synthesis of surface glycoproteins relevant to cellular activation and proliferation, but confirming this will require analysis of more genes. This regulatory site is also found frequently in primate viruses. Although the role of NF-κB in retroviral replication and leukaemogenesis is not completely understood, HTLV-I and HIV-1 use this class of transcription factors in different ways. Our data suggest that an NF-κB-like factor is a target of HTLV-I infection, activated by the *tat-I* product. In the case of T cells infected with HIV, NF-κB is not a target of viral transactivation but a cellular activator of HIV gene expression. In uninfected cells, it may regulate expression of a growth factor receptor, IL-2Rα.

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## Transcript localization of four opsin genes in the three visual organs of *Drosophila*; RH2 is ocellus specific

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*Drosophila* and other Dipteran flies have three different kinds of visual organs; in the adult a pair of compound eyes<sup>1</sup> and three dorsal ocelli<sup>2,3</sup>; and in the larva a pair of internal photoreceptor organs<sup>4,5</sup>. They develop in distinct ways, yet have certain features in common. All three organs use retinal-derived chromophores, coupled to distinct opsins, to provide a diversity of spectral sensitivities. Four opsin genes have been identified thus far in *Drosophila*: Rh1, Rh2, Rh3 and Rh4 (refs 6–11). We have used *in situ* hybridization to study the messenger RNAs expressed by these four opsin genes in all three visual organs. Rh1, Rh3 and Rh4 are already known to be expressed in different subsets of cells in the compound eye<sup>8–10</sup>. We found that, in contrast, opsin Rh2 is the predominant opsin expressed in the ocelli. Opsin Rh1 is known to be expressed in the larval photoreceptor<sup>12</sup>. We found that Rh3 and Rh4 are as well, but not Rh2. The ocellar-specific gene expression of Rh2 is of particular interest for its possible bearing on the function of the ocellus<sup>13,14</sup>.

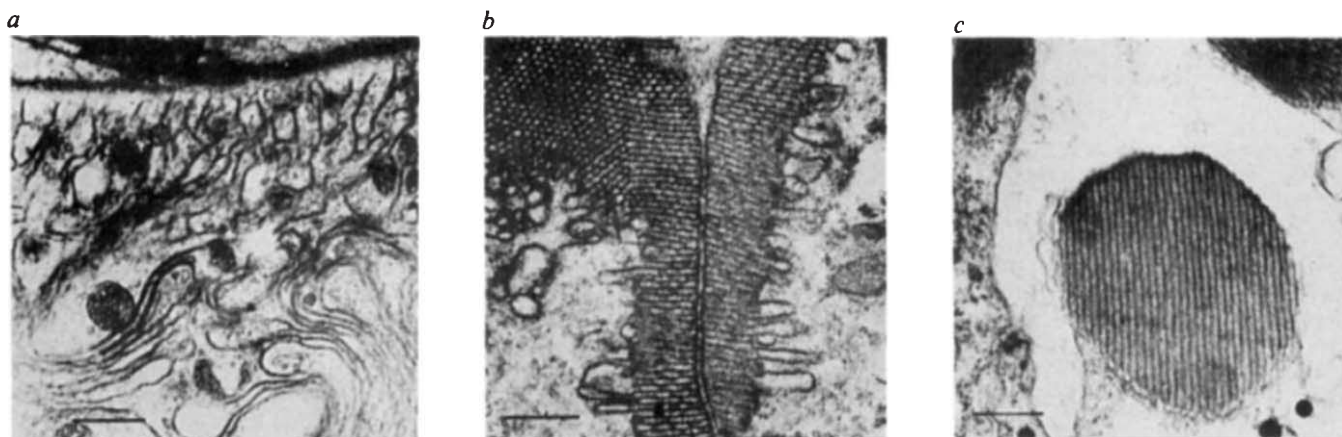
In *Drosophila*, a bilateral pair of larval photoreceptor organs (Bolwig's organs)<sup>4</sup> becomes evident about midway through embryogenesis near the anterior end of the embryo<sup>15</sup>. In the newly hatched larva, this organ is located within the cephalopharyngeal skeleton below the protocerebral bridge as described for *Musca*<sup>4</sup>. While ordinarily difficult to identify, it is clearly delineated by monoclonal antibodies such as MAb24B10<sup>16,17</sup>, which show it to consist of a dozen photoreceptor neurons. Their axon bundle extends posteriorly and passes through the eye-antennal imaginal disc and its optic stalk, terminating deep in the larval brain. The cell membranes elaborate twisted microvilli (Fig. 1a), resembling the rhabdomeric structures found in evolutionally primitive creatures<sup>18,19</sup>. A strongly negative phototactic behaviour of the larva is mediated by this organ<sup>4,5</sup>. During the pupal stage, as the adult ocelli and the

compound eyes form, Bolwig's organ is histolysed, and its nerves degenerate.

In the adult fly, each of the three ocelli on the top of the head has a single light-collecting lens, about 40  $\mu\text{m}$  in diameter, and a layer of some 90 photoreceptor cells with fairly well organized rhabdomeric microvilli (Fig. 1b) on the distal portion of each cell<sup>20</sup>. The ensemble is surrounded by pigment cells<sup>2</sup>. Axon bundles from the three ocelli converge on a ganglion, synapsing on the apical dendrites of 12 second-order neurons and an unknown number of small interneurons<sup>2,3</sup>. The 12 secondary neurons form two bundles of six axons that feed into the two sides of the brain. Some of the fibres cross over, so that each side of the brain receives inputs from all three ocelli<sup>2,21</sup>.

The adult compound eye is an exquisitely regular structure made up of some 750 ommatidia<sup>22</sup>, each containing eight precisely arranged photoreceptor neurons with long rhabdoms composed of neat stacks of microvilli (Fig. 1c). The photoreceptor neurons are of three main types, as defined by genetic, physiological and anatomical criteria: cells R1–R6, cell R7 and cell R8 (refs 1, 22, 23). Photoreceptor cells R1–R6 express a common rhodopsin, Rh1 (refs 6, 7) and direct their axons to similar synaptic targets in the first optic ganglion (the lamina)<sup>21,24</sup>. The R1–6 cells are specifically affected by certain genetic defects, such as mutations in the gene *ninaE*, the structural gene for opsin Rh1 (refs 6, 7). Photoreceptor neuron R7, different in anatomy and spectral sensitivity, is specifically affected by the genetic lesion *sevenless*, which precisely removes cell R7 (refs 23, 25, 26). Subsets of the R7 cells express one or the other of two opsin genes, Rh3 or Rh4 (refs 9–11). Photoreceptor R8 has a spectral sensitivity different from those of R1–R6 and R7 (refs 23, 27). Cowman *et al.*<sup>8</sup> reported on an opsin gene Rh2, suggesting that it may be expressed in cell R8. Both R7 and R8 send their axons through the first optic ganglion without synapsing there, to terminate in separate layers in the second optic ganglion (the medulla). The compound eye is involved in an extensive repertoire of behavioural responses, including phototaxis and pattern recognition<sup>1</sup>.

The expression of at least some of the opsin genes does not appear to be restricted to the compound eyes. Mismar and Rubin<sup>12</sup>, using flies transformed with an Rh1 promoter-driven reporter gene ( $\beta$ -galactosidase), detected expression of the reporter in a subset of cells in the larval photoreceptor organ, in addition to the expected expression in the retina.



**Fig. 1** Transmission electron micrographs of the photoreceptive membranes in the three *Drosophila* visual organs. *a*, Larval photoreceptor organ in first instar larva. This transverse section reveals the elaborate foldings of membranes on the outer surfaces of the photoreceptor cells. Bar = 0.5  $\mu\text{m}$ . *b*, Adult ocellus (one day post emergence). This section, tangential to the surface of the ocellus, shows the organization of the rhabdomeric microvilli. Bar = 0.5  $\mu\text{m}$ . *c*, Adult compound eye (one day post emergence). This section, tangential to the surface of the retina, reveals the highly ordered rhabdomeric microvilli of these photoreceptor cells. Bar = 0.5  $\mu\text{m}$ . The electron microscopy was performed as described in ref. 22.

**Fig. 2** Expression *in situ* of four opsin genes in the *Drosophila* ocellus. Wildtype flies (strain Oregon-R) were sectioned parallel to the dorsal head surface, cutting through photoreceptor cell layers of all three ocelli. Each pair of photographs shows phase-contrast and dark-field views of a 6  $\mu\text{m}$  cryostat section hybridized with a DNA probe derived from one of the four opsin clones. For a given probe, both the ocellar section of Fig. 2 and the compound eye section of Fig. 4 are from the same fly head, serially sectioned and processed on the same microscope slide. *a, b*, Opsin Rh1 probe. No Rh1 mRNA is evident in the receptor layers of the ocelli (OR1, OR2, OR3). The signal associated with the cuticle is due to non-specific sticking of the probe to chitinous structures (C)<sup>28</sup>. The probe specific activity was  $3 \times 10^7$  c.p.m.  $\mu\text{g}^{-1}$  DNA, and the slide was exposed for 4 d. *c, d*, Opsin Rh2 probe. There are abundant transcripts in each of the three ocelli. Probe specific activity  $4 \times 10^7$  c.p.m.  $\mu\text{g}^{-1}$  DNA; exposure 5 d. *e, f*, Opsin Rh3 probe. No signal is evident. Probe specific activity  $5 \times 10^7$  c.p.m.  $\mu\text{g}^{-1}$  DNA; exposure 7 d. *g, h*, Opsin Rh4 probe. No signal is evident. Probe specific activity  $2 \times 10^7$  c.p.m.  $\mu\text{g}^{-1}$  DNA; exposure 7 d. OR, Ocellar photoreceptor layer; C, cuticle. Bar = 20  $\mu\text{m}$ .

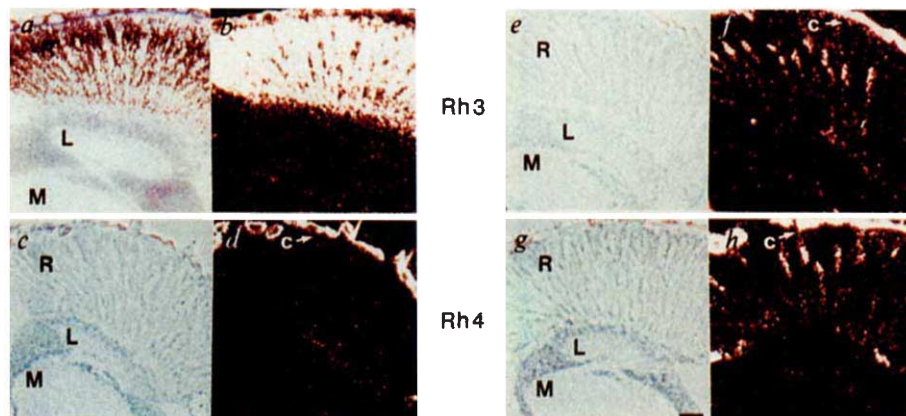
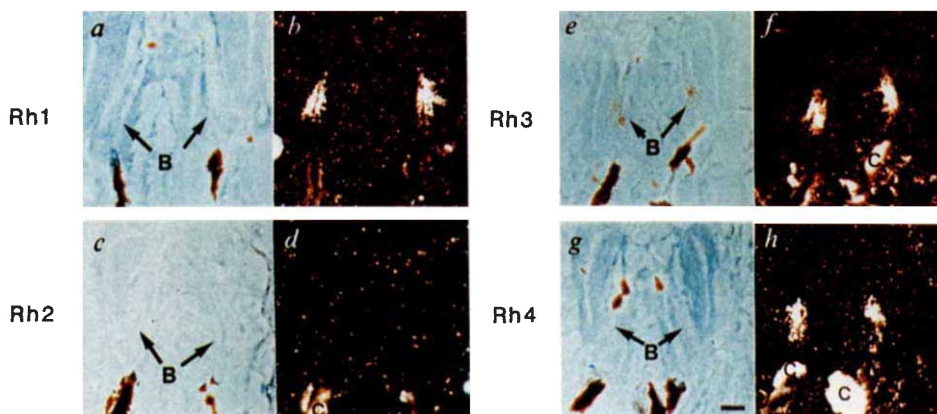
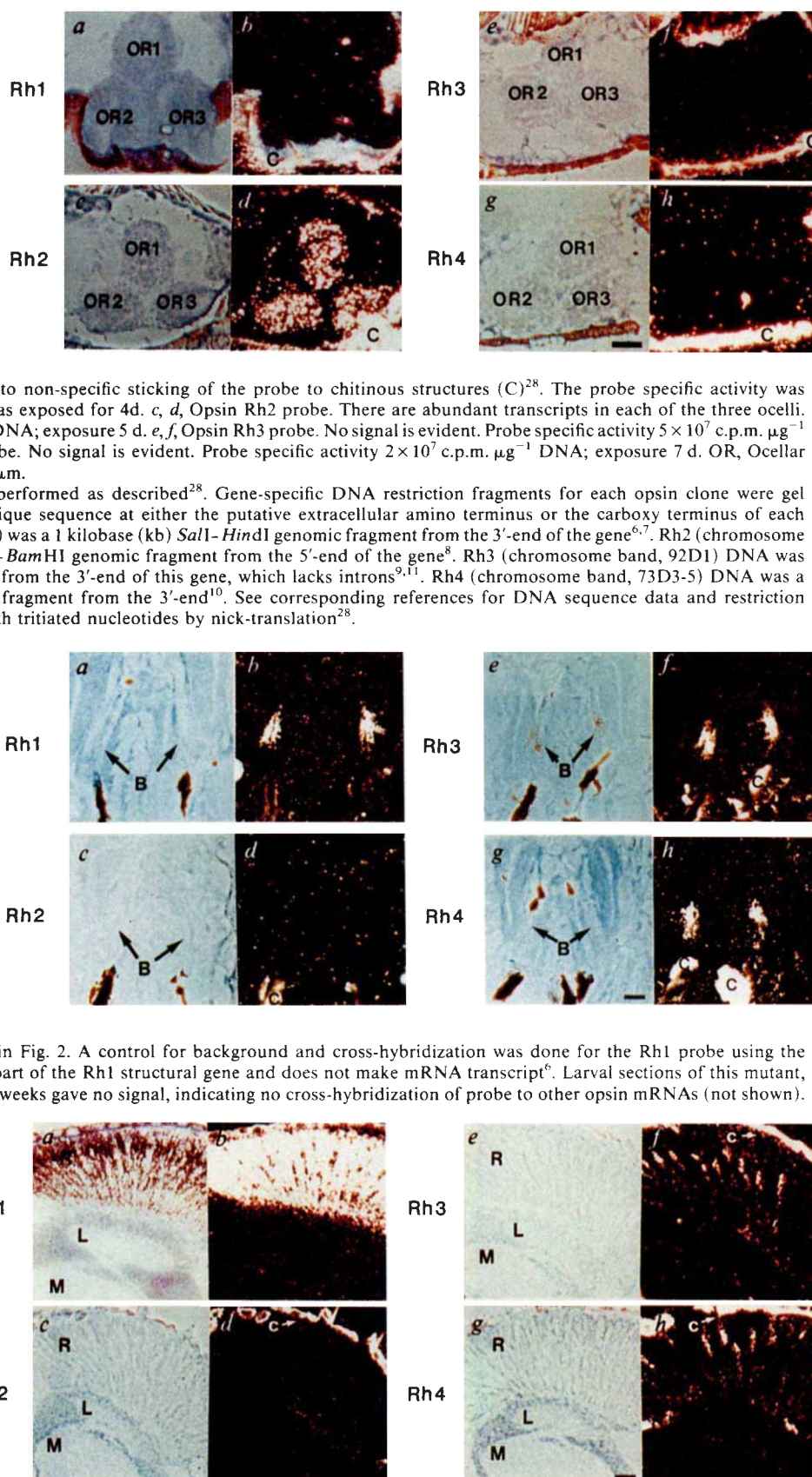
**Methods.** The hybridization *in situ* was performed as described<sup>28</sup>. Gene-specific DNA restriction fragments for each opsin clone were gel purified. Each fragment came from a unique sequence at either the putative extracellular amino terminus or the carboxy terminus of each protein. Rh1 (chromosome band, 92B6-11) was a 1 kilobase (kb) *SalI-HindI* genomic fragment from the 3'-end of the gene<sup>6,7</sup>. Rh2 (chromosome band, 91D1-2) DNA was a 0.4 kb *EcoRI-BamHI* genomic fragment from the 5'-end of the gene<sup>8</sup>. Rh3 (chromosome band, 92D1) DNA was a 0.8 kb *PstI-HindIII* genomic fragment from the 3'-end of this gene, which lacks introns<sup>9,11</sup>. Rh4 (chromosome band, 73D3-5) DNA was a 0.4 kb *PstI-EcoRI* complementary DNA fragment from the 3'-end<sup>10</sup>. See corresponding references for DNA sequence data and restriction maps<sup>11-16</sup>. DNA probes were labelled with tritiated nucleotides by nick-translation<sup>28</sup>.

**Fig. 3** Expression *in situ* of four opsin genes in the larval photoreceptor organ. Wild-type, 1-d-old, third-instar larvae were used. Cryostat sections (6  $\mu\text{m}$ ) cut transversely through the cephalopharyngeal apparatus reveal bilateral symmetry of the two groups of photoreceptor cells. Each pair of photographs shows phase-contrast and dark-field views of a single section. *a, b*, Opsin Rh1 probe. This mRNA is abundantly expressed. Exposure 20 d. *c, d*, Opsin Rh2 probe. No *in situ* hybridization signal is evident. Exposure 20 d. *e, f*, Opsin Rh3 probe. Positive signal indicates presence of Rh3 transcript. Exposure 20 d. B, Bolwig's organ; C, Chitinous mouth parts. Bar, 20  $\mu\text{m}$ .

**Methods.** Probe specific activities are as in Fig. 2. A control for background and cross-hybridization was done for the Rh1 probe using the *ninaE* mutant, Df(3R)0117, which lacks part of the Rh1 structural gene and does not make mRNA transcript<sup>6</sup>. Larval sections of this mutant, probed with Rh1 and exposed for up to 4 weeks gave no signal, indicating no cross-hybridization of probe to other opsin mRNAs (not shown).

**Fig. 4** Expression *in situ* of four opsin genes in the adult *Drosophila* compound eye. Horizontal 6  $\mu\text{m}$  cryostat sections of wild-type flies reveal the retina, the lamina and the medulla. Each pair of photographs shows phase-contrast and dark-field views of a single section. For a given probe, both the ocellar section of Fig. 2 and the compound eye section of Fig. 4 are from the same fly head, serially sectioned and processed on the same microscope slide. *a, b*, Opsin Rh1 probe. This mRNA is abundant throughout the depth of the retina. *c, d*, Opsin Rh2 probe. No *in situ* hybridization signal is evident. *e, f*, Opsin Rh3 probe. The signal is restricted to a subset of photoreceptor cells in the distal retina. Mutant *sevenless* compound eyes, probed with Rh3 and Rh4 gave no signal after exposures of up to 3 weeks, indicating that the normal expression of Rh3 is R7-specific (data not shown). *g, h*, Opsin Rh4 probe. This signal is also restricted to be a subset of R7 photoreceptor cells, shown by previous studies to be a different subset from the Rh3 positive cells<sup>9,10</sup>. R, Retina; L, Lamina (first optic ganglion); M, Medulla (second optic ganglion); C, Cuticle or lens. Bar = 20  $\mu\text{m}$ .

**Methods.** Probe specific activities and exposure times are the same as in Fig. 2. A control for background and cross-hybridization was done for the Rh1 probe using the *ninaE* mutant, Df(3R)0117 (see Fig. 3). Compound eye sections of this mutant, probed with Rh1 and exposed for up to 3 weeks, gave no specific signal, indicating no cross-hybridization of the probe to other opsin mRNAs.





Our interest was to determine which, if any, of the four known opsin genes are expressed in the ocellus and in the larval photoreceptor organ, by localizing the mRNA transcripts *in situ*. DNA restriction fragments representing sequences unique for each opsin (given to us by Charles Zuker) were radiolabelled with tritiated nucleotides and hybridized to cryostat sections of wild-type and mutant compound eyes, ocelli and larval photoreceptors.

Figure 2 shows autoradiograms of the ocelli in wild-type flies indicating the localization of each of the four *Drosophila* opsin mRNAs. Our results demonstrate that Rh2 mRNA is abundantly expressed in the ocellus (Fig. 2c, d), while Rh1, Rh3 and Rh4 are not. For each opsin probe, the experiment was performed three times, using three separate nick-translation reactions and three or more serially sectioned wild-type heads each time. The exposures in each experiment ranged from a few days to several weeks. Actual exposure times and probe specific activities for the examples shown are included in the legend to Fig. 2. The lack of Rh1 expression in the ocellus is consistent with the results of Mismar and Rubin<sup>12</sup>, who did not detect Rh1 promoter activity in ocelli of transformed flies.

The transcript localization of the opsin genes in the larval photoreceptor organ is shown in Fig. 3. The autoradiograms indicate that Rh1, Rh3 and Rh4 are expressed in these simple photoreceptor cells while Rh2 is not. At least eight individual, serially sectioned, third instar larvae were hybridized with each probe, and all gave consistent results. The Rh1 transcript expression observed in the larval photoreceptor is in agreement with the results of Mismar and Rubin<sup>12</sup> who, using a reporter gene in transformed larvae, detected Rh1 promoter activity in a subset of the cells of this organ. Our observations show that the Rh3 and Rh4 genes are transcribed in this organ although the resolution of the *in situ* technique does not permit us to assign the opsins to specific cells.

Figure 4 shows, for comparison, the expression of the four opsin genes in sections of the wild-type compound eye. For each probe, the same individual head was used as for the ocelli in Fig. 2. The expression of Rh1, Rh3, and Rh4 is consistent with previously reported findings<sup>8-11</sup>. In contrast, no Rh2 signal is evident (Fig. 4c, d) in the same head that reveals a strong signal in the ocelli (Fig. 2c, d).

With much longer (threefold) exposure times, the Rh2 probe produces a weak signal in the proximal adult retina, in addition to areas in the brain (data not shown). This raises the question of whether there may be some low level of Rh2 expression in the retina, as reported by Cowman *et al.*<sup>8</sup> Using an Rh2 probe on Northern blots of mRNA purified from heads of mutant flies lacking photoreceptor cells R1-6 (*ora*) or R7 (*sevenless*), they found a normal intensity of the Rh2 transcript, and therefore suggested that Rh2 was expressed in cell R8. In those mutants, however, the ocelli were intact and could have been responsible for the strong Rh2 transcript signal. While the possibility of low level expression of Rh2 in R8 cells is not excluded, there are two caveats to this conclusion. On long exposures, a signal could result from cross-hybridization of the Rh2 probe, even at low efficiency, to mRNA of opsins other than Rh2. Another possible artifact could be the commonly observed non-specific sticking of many DNA probes to cuticular and tracheal structures<sup>28</sup>, as is evident in Figs 2, 3 and 4. The compound eye and brain have extensive arrays of fine, chitinous tracheoles<sup>21,29</sup>. These can readily be seen by epi-illumination with 365 nm light on sectioned *Drosophila* tissue revealing in the retina a radial, spoked pattern, strongest in the proximal portion, in addition to scattered streaks in the brain. These patterns resemble the weak Rh2 hybridization signal detected in the retina with long exposures. In any case, the specific activity of the Rh2 signal is clearly many fold greater in, if not unique to, the ocellus.

The observation of Rh2 transcripts in the ocellus is supported by the related studies of Mismar *et al.*<sup>30</sup>. Flies transformed with an Rh2 promoter-driven reporter gene ( $\beta$ -galactosidase) showed

**Table 1** Normal expression of opsin mRNA transcripts, *in situ*, in the three kinds of *Drosophila* visual organs.

| Opsin | Chromosome band | mRNA present in      |         |              |
|-------|-----------------|----------------------|---------|--------------|
|       |                 | Larval photoreceptor | Ocellus | Compound eye |
| Rh1   | 92B             | +                    | -       | +            |
| Rh2   | 91D             | -                    | +       | -            |
| Rh3   | 92D             | +                    | -       | +            |
| Rh4   | 73D             | +                    | -       | +            |

Chromosome band refers to the cytological position of the gene on the salivary gland polytene chromosome<sup>6-11</sup>. In the compound eye, expression of Rh1 is restricted to photoreceptor cells R1-R6 (refs 6, 7) and Rh3 and Rh4 are restricted to non-overlapping sets of photoreceptor cell (refs 9-11). In the larval photoreceptor organ, the level of resolution of *in situ* hybridization did not permit the identification of unique cell types.

expression of the reporter in the ocelli, but not in the retina or the larval photoreceptor. In flies transformed by a chimaeric gene with the Rh1 promoter linked to the Rh2 structural gene, Zuker *et al.*<sup>31</sup> found the Rh2 photopigment to be expressed in the R1-R6 cells, where it is not normally present. Feiler *et al.*<sup>32</sup> have also done spectroscopic and Northern blot analysis on such transformed flies, demonstrating that Rh2 is the major ocellar photopigment. These observations are consistent with our *in situ* hybridization results. Table 1 summarizes the sites of expression of the four known opsin genes in the three visual organs.

In *Drosophila*, the three visual organs contain certain common antigens, recognizable by monoclonal antibodies, indicating that some genes are expressed in all three. For example, monoclonal antibody MAb24B10<sup>16</sup>, recognizes a photoreceptor cell-specific membrane protein, chaoptin, which is found in all three visual organs<sup>17</sup>, and has been shown to be required for normal morphogenesis of the adult rhabdomeres<sup>33</sup>. An interesting question is whether, in addition to a set of genes essential for all three photosystems, there are genes that are specifically transcribed in each photosystem. Rh2 would appear to be such a gene. The presence of transcripts of opsins Rh1, Rh3 and Rh4 in the larval photoreceptor, combined with the Rh1 promoter activity found by Mismar and Rubin<sup>12</sup>, is consistent with the observation that larvae which lack Rh1 still exhibit negative phototaxis<sup>5</sup>. The larval photoreceptor may be made up of distinct cell types, as is the compound eye. It will be of interest to determine the specific organization of these cells and to determine which of the mutations that affect the compound eye also affect Bolwig's organ.

The spectral sensitivity of the ocellus appears to be different from those of the compound eye cell types<sup>14</sup>. Electroretinograms of the ocellus, combined with adaptation studies using different wavelengths, suggest that there is only one photopigment, with maximal sensitivity in the near UV and blue, that is responsible for its electrical response<sup>14</sup>. The predominant use of Rh2 in the ocellus is presumably related to its function. It is unlikely that the ocellus is used for image formation, as the focal plane of its lens lies far below the level of its rhabdomeres<sup>7</sup>. The wiring of the three ocelli to the brain<sup>2,21</sup>, however, and their responsiveness to small changes in light intensity<sup>14</sup>, may allow the trio to act as a simple detector for shadow motion and direction. The maximal sensitivity to blue and ultraviolet light might serve this purpose well under the blue sky of the normal environment.

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## Inhibition of bacterial ice nucleators by fish antifreeze glycoproteins

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Certain bacteria promote the formation of ice in super-cooled water by means of ice nucleators which contain a unique protein associated with the cell membrane<sup>1</sup>. Ice nucleators in general are believed to act by mimicking the structure of an ice crystal surface, thus imposing an ice-like arrangement on the water molecules in contact with the nucleating surface and lowering the energy necessary for the initiation of ice formation<sup>2</sup>. Quantitative investigation of the bacterial ice-nucleating process has recently been made possible by the discovery of certain bacteria that shed stable membrane vesicles with ice nucleating activity<sup>3</sup>. The opposite effect, inhibition of ice formation, has been described for a group of glycoproteins found in different fish and insect species<sup>4,5</sup>. This group of substances, termed antifreeze glycoproteins (AFGPs), promotes the supercooling of water with no appreciable effect on the equilibrium freezing point or melting temperature<sup>6</sup>. Substantial evidence now indicates that AFGPs act by binding to a growing ice crystal and slowing crystal growth<sup>7,8</sup>. As the ice-nucleating protein surface is believed to have a structure similar to an embryonic ice crystal, AFGPs might be predicted to interact directly with a bacterial ice-nucleating site. We report here that AFGPs from the antarctic fish *Dissostichus mawsoni* inhibit the ice-nucleating activity of membrane vesicles from the bacterium *Erwinia herbicola*. The inhibition effect shows saturation at high concentration of AFGP and conforms to a simple binding reaction between the AFGP and the nucleation centre.

The drop freezing assay<sup>9,10</sup> is generally used to characterize populations of ice nucleators. The solution containing the ice nucleators is first divided into a number of small drops and their freezing temperature is then recorded on cooling. The number of drops frozen at any temperature enables calculation

of the differential nucleator concentration<sup>9</sup>  $k(\Theta)$ , that is, the concentration of nucleators which are active at any given temperature  $\Theta$ . Figure 1a shows how the number of 1  $\mu$ L drops frozen varies with temperature for three different dilutions of ice-nucleating vesicles from *Erwinia herbicola*. The distribution of active nucleators is calculated from the slope of these data, and is reported in Fig. 1b.

The influence of AFGP on the population of active nucleators is shown in Fig. 2. In solutions with fixed total concentrations of nucleators, the concentration of nucleators active at any given temperature decreases with increasing AFGP concentration. Strikingly, however, increasing the AFGP concentration beyond a certain point produces no further change in the spectrum of active nucleators. Similar inhibition results with AFGPs were found with other bacterial nucleators, such as an ice nucleating strain of *Pseudomonas syringae*. In contrast, no inhibition of ice-nucleation was found using a mixture (250  $\mu$ g ml<sup>-1</sup> each) of the following proteins with indicated relative molecular masses ( $M_r$ ): oxytocin (1,000),  $\alpha$ -lactalbumin (14,400), soybean trypsin inhibitor (22,700), and  $\beta$ -lactoglobulin (35,000). Additionally,

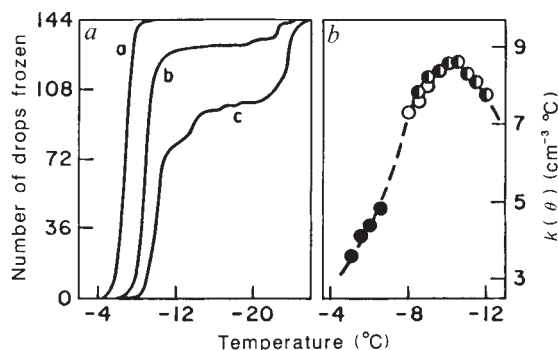
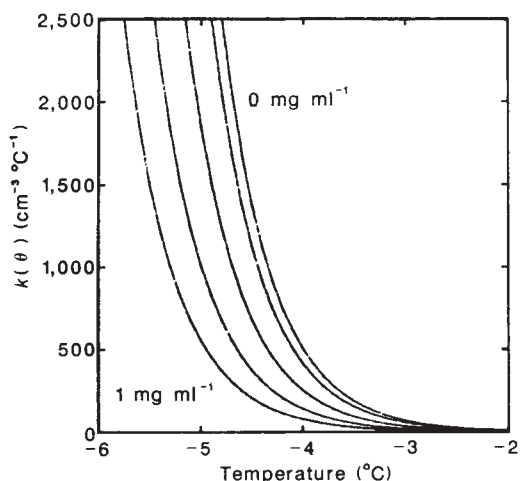


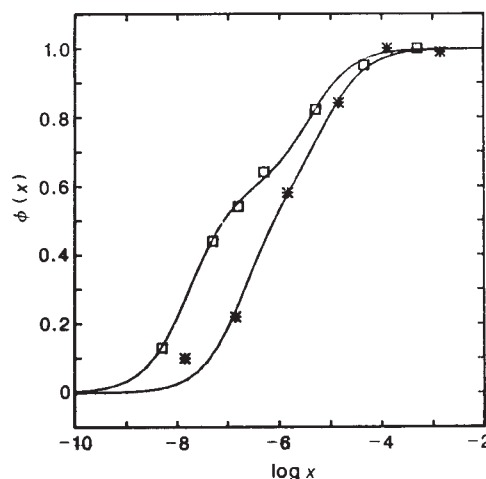
Fig. 1 a, Distribution of freezing events with temperature in drop freezing assays of *Erwinia herbicola* ice-nucleating vesicle solutions. The original preparation was diluted  $10^2$  (curve a),  $10^3$  (curve b) and  $10^6$  (curve c) times. Ice-nucleating vesicles were purified from *Erwinia herbicola*<sup>3</sup> and suspended in 10 mM Tris, 10 mM MgCl<sub>2</sub>, pH 7.4. The concentration of nucleators active at any given temperature  $\Theta$ , was calculated as follows<sup>9</sup>:  $k(\Theta) = [1/(V \cdot N(\Theta))] dN(\Theta)/d\Theta$ , where  $dN(\Theta)$  is the number of 1  $\mu$ L drops frozen in the temperature interval  $d\Theta$ ,  $V$  is the volume of the drops, and  $N(\Theta)$  the number of drops still unfrozen at the temperature  $\Theta$ . b, Differential nucleator concentrations in the original preparation calculated using the lower halves of curves a (●), b (○) and c (●) in (a) and multiplying the results of the above equation by the dilutions made.

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**Fig. 2** Differential nucleator spectra of ice-nucleating vesicles ( $\sim 1.5 \times 10^3 \text{ ml}^{-1}$ ,  $-4.5^\circ\text{C}$ ), for different concentrations of AFGP in 10 mM Tris, 10 mM  $\text{MgCl}_2$ , pH 7.4. The AFGPs were isolated from the antarctic fish *Dissostichus mawsoni* and purified by ion-exchange chromatography. AFGPs from this fish separate into eight components ranging in  $M_r$  from 2,500 to 32,000, and are numbered from 1 to 8 with decreasing  $M_r$ .<sup>6</sup> Two different mixtures of AFGP were used, a high molecular weight preparation containing components 2 and 3, and a polydisperse fraction containing all components 1–8. The curves depict the effect of the polydisperse mixture of AFGP at various concentrations. At concentrations  $>1 \text{ mg ml}^{-1}$  the results were indistinguishable from the  $1 \text{ mg ml}^{-1}$  curve. The results of adding a  $1 \text{ mg ml}^{-1}$  mixture of other proteins (oxytocin,  $\alpha$ -lactalbumin, soybean trypsin inhibitor,  $\beta$ -lactoglobulin) with no antifreeze activity spanning a  $M_r$  range of 1,000–35,000 were identical with the curve obtained in the absence of AFGP.



**Fig. 3** Fractional inhibition  $\phi(x)$  of  $-4.5^\circ\text{C}$  nucleators as a function of AFGP average molar concentration,  $x$ . The composition of the polydisperse fraction (\*) is predominantly low  $M_r$  AFGP with an average  $M_r \sim 7,000$ . The high  $M_r$  fraction (□) is characterized by a relatively narrow weight distribution with an average  $M_r$  of 20,000. The data of fractional inhibition as a function of concentration for the high  $M_r$  AFGP sample show heterogeneity, implying at least two classes of binding sites with different affinities for the high  $M_r$  material. The shifted positions of the data points obtained with the polydisperse (predominantly low  $M_r$ ) sample indicates both that the average affinity for the low  $M_r$  AFGP is less than that for the high  $M_r$  AFGP and that the affinity of the low  $M_r$  AFGP is similar for both classes of ice nucleation binding sites. A simple model based on two sites for the high  $M_r$  AFGP and a single site for the low  $M_r$  was used to generate the smooth curves shown.

no inhibition was seen with the following glycoproteins ( $250 \mu\text{g ml}^{-1}$ ):  $\alpha_1$ -acid glycoprotein, chorionic gonadotropin, fetuin, haptoglobulin, immunoglobulin A and thyroglobulin. This implies that the inhibition by AFGP is specific. Samples containing only AFGP and no added ice-nucleators did not freeze above  $-15^\circ\text{C}$ .

The observation that inhibition reaches a maximum at high AFGP concentration suggests that AFGP interacts directly with ice nucleators according to mass law binding, in which case a degree of inhibition can be calculated as a function of AFGP concentration. At any given temperature  $\Theta$  the fractional inhibition  $\phi(x)$  was computed as a function of the AFGP concentration,  $x$ , as follows

$$\phi(x) = \frac{k_0(\Theta) - k_x(\Theta)}{k_0(\Theta) - k_\infty(\Theta)} \quad (1)$$

where  $k_0$  and  $k_\infty$  are the differential nucleator concentrations in the absence of AFGP and in the presence of saturating AFGP concentrations, and  $k_x$  is the value of  $k$  when the AFGP concentration is  $x$ , at any given temperature  $\Theta$ . In Fig. 3 the results are shown as fractional inhibition versus the AFGP concentration at  $-4.5^\circ\text{C}$ . At this temperature, for the concentration of nucleators used, all parameters in equation 1 were accurately determined. The data presented in this form reveal the saturation effect expected from mass law binding of AFGP to ice nucleators.

The inhibition of ice-nucleation depends upon the relative molecular mass of the AFGP (Fig. 3). The higher molecular mass AFGP is the more effective inhibitor, that is, it has a higher binding affinity. Presumably the larger the size of the AFGP, the greater the number of interactions with the ice nucleator site. There is a noticeable heterogeneity in the data from the

high relative molecular mass AFGP—its binding data are spread over a broader range of concentrations than would be predicted for binding to a single site. The lack of heterogeneity observed with the AFGP of low relative molecular mass suggests that all the ice nucleator sites which can interact with the AFGP may be equally accessible to smaller AFGP molecules. The ability of AFGP to disrupt nucleation by ice-nucleating vesicles indicates that there are nucleator sites exposed on the outer surface of these membrane vesicles. The data in Fig. 2, however, show that complete inhibition of ice nucleation cannot be achieved even at high concentrations of AFGP. Presumably the sites that remain active are contained within the vesicle, and are not accessible to proteins in the external solution.

The molecular architecture of bacterial ice nucleators is unknown, but is thought to include a self-associating membrane protein, 20–40 subunits of which could aggregate to nucleate ice formation at  $-4.5^\circ\text{C}$  (ref. 11). We conclude that the binding of AFGP is specific to the nucleation site, whatever its nature, and that it results in the disruption of the ordered array of water molecules necessary for nucleation of ice crystals.

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## Contribution of hydrophobic interactions to protein stability

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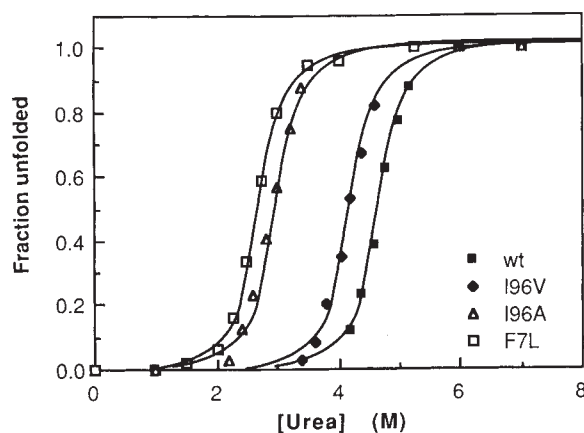
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A major factor in the folding of proteins is the burying of hydrophobic side chains. A specific example is the packing of  $\alpha$ -helices on  $\beta$ -sheets by interdigitation of nonpolar side chains. The contributions of these interactions to the energetics of protein stability may be measured by simple protein engineering experiments. We have used site-directed mutagenesis to truncate hydrophobic side chains at an  $\alpha$ -helix/ $\beta$ -sheet interface in the small ribonuclease from *Bacillus amyloliquefaciens* (barnase). The decreases in stability of the mutant proteins were measured by their susceptibility to urea denaturation. Creation of a cavity the size of a  $-\text{CH}_2-$  group destabilizes the enzyme by  $1.1 \text{ kcal mol}^{-1}$ , and a cavity the size of three such groups by  $4.0 \text{ kcal mol}^{-1}$ .

Protein design *de novo* requires a quantitative understanding of the rules for the folding of proteins (see ref. 1 for recent review). One approach is the direct application of computational methods. Important advances are being made as methods improve and computational power increases. A complementary approach is descriptive, surveying known structures and formulating general rules about motifs. An important example is the classification of packing of helices and sheets in proteins<sup>2-7</sup>. There is, however, no direct evidence on the strengths and specificity of the interactions involved and all estimates have been made from model compounds. Direct, simple experimental data on proteins are required in general to test and refine predictive methods.

Significant experimental contributions are now being made using protein engineering (see, for example, refs 8, 9). Work so far has primarily used the selection of temperature-sensitive mutants after random mutagenesis and the construction of large numbers of mutations at the temperature-sensitive loci. We are using an alternative strategy: adopting methods previously employed to dissect the structure and activity of the tyrosyl-tRNA synthetase<sup>10</sup> to provide a quantitative experimental basis to the descriptive approach of refs 2-7. Mutations that remove defined interactions without introducing new or unfavourable interactions, that is, nondisruptive deletions<sup>11</sup> are made on the basis of the crystal structure of the wild-type enzyme. It is especially important to use nondisruptive deletions in analysing protein folding because the free energy of unfolding depends on the difference in free energy between the folded and denatured states and so both should be perturbed as little as possible. Empirical measurements of the energetics of interactions are obtained that may be used directly for experimental design and provide raw data for refinement of computational methods and theory.

The enzyme we have chosen for these studies is barnase, the RNase from *B. amyloliquefaciens*<sup>12</sup>. This enzyme has almost ideal characteristics as a paradigm for protein folding for the following reasons. It is a monomer of 110 residues of  $M_r$  12,382, one of the smallest known enzymes. The crystal structure has been solved at high resolution<sup>13</sup>; the small size and favourable crystals enabling the resolution to be increased to  $1.4 \text{ \AA}$ . In addition, barnase is small enough for sequence specific two-dimensional NMR assignments to be made and to be tractable to analysis by computational methods. Yet it is large enough to have significant secondary structure. It has, for example, the packing of an  $\alpha$ -helix on a  $\beta$ -sheet<sup>13</sup>: the helix formed from



**Fig. 1** Urea denaturation of recombinant barnase and engineered mutants. Denaturation was observed spectroscopically using difference spectroscopy at 286 and 270 nm. Experiments were performed at  $25^\circ\text{C}$  in a buffer containing 19.3 mM MES acid and 30.7 mM base (MES = 2-(*N*-morpholino) ethanesulphonic acid) at ionic strength 0.05 M and pH 6.30 (except for Phe  $\rightarrow$  Leu7 which was at pH 6.15). The stability of the protein hardly varies between pH 6.0 and 6.5 under these conditions. The concentration of barnase was between 9 and  $12 \mu\text{M}$ . There is a decrease of 13.5% in absorbance at 286 nm and an increase of 4.5% at 270 nm on unfolding ( $\Delta A_{286} - \Delta A_{270}$ ) was measured since this both amplifies the signal and minimizes dilution errors as  $A_{286} \sim A_{270}$ . The solid curves are just for visual aid.

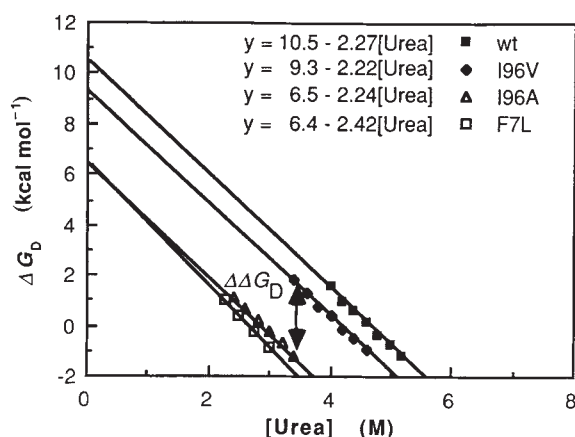
**Methods.** Wild-type enzyme was secreted from *E. coli* harboring the plasmid pMT410, a derivative of pUC9 (ref. 16 and R. W. Hartley, personal communication). This construction contains the structural gene for barnase fused to the alkaline phosphatase promoter and signal sequence and the gene for barstar (the polypeptide inhibitor of barnase) under the control of its own promoter contained on a 1.4 kilobase (kb) *EcoRI*-*HindIII* fragment. For mutagenesis, this fragment was subcloned into M13. The mutated genes were subsequently cloned back into pUC9 for expression. Site-directed mutagenesis was carried out using the double primer method<sup>20</sup>. The mutagenic primers were used together with an M13 universal primer. To improve the yield of mutants, a strain defective in mismatch repair, *E. coli* BMH 71-18 *mut L* (ref. 27) was used in the transfection. Mutant bacteriophages were identified by oligonucleotide hybridization<sup>28</sup> and the mutations verified by dideoxy sequencing of the entire genome. The following primers were used to direct the mutations: 5'TTTTGTAG\*AC\*GAGCCAG3', Ile  $\rightarrow$  Val96; 5'TTTTGTAG\*AC\*GAGCCAG3' Ile  $\rightarrow$  Ala96; Phe  $\rightarrow$  Leu7 5'CCCCGTCAG\*CGTGTG3' (asterisks follow mismatches). Cells were grown in a low phosphate medium<sup>29</sup>. Secreted wild-type and mutant enzymes were purified to homogeneity (D. Mossakowska, K.N. and A.R.F., in preparation).

residues 6-18 packs against the anti-parallel  $\beta$ -sheet formed from residues 50-55, 70-75, 85-91, 94-101 and 106-108. The protein is composed of one domain and undergoes a reversible thermal or urea-induced denaturation, approximating to a two-state process<sup>14,15</sup>. This enables thermodynamic measurements to be made on the factors that influence protein folding and stability. Equilibrium and kinetic measurements of denaturation may readily be made by difference spectroscopy. Barnase has no disulphide bridges. This reduces structural constraints in the denatured protein and so decreases complications in analysing denaturation. The protein has been cloned and expressed<sup>16</sup>.

The mutations chosen in this study are the truncations Ile96  $\rightarrow$  Val, Ile96  $\rightarrow$  Ala and Phe7  $\rightarrow$  Leu. The first two mutations are, in theory, true nondisruptive deletions since simple truncations are involved. Phe  $\rightarrow$  Leu is a conventional conservative mutation, but there is a change in geometry about the  $\gamma$ -carbon ( $sp^2$  in Phe and  $sp^3$  in Leu). The mutations remove hydrophobic inter-

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**Fig. 2** Linear dependence of  $\Delta G_D$  on [urea] over experimental ranges allows simple determination of  $\Delta G_D^{H_2O}$  and  $\Delta\Delta G_D$ . The value of  $\Delta\Delta G_D$  between, for example, Ile  $\rightarrow$  Val96 and Ile  $\rightarrow$  Ala96, may readily be read off the graph at an intermediate concentration of urea (such as 3.55 M) to be 2.9 kcal mol $^{-1}$ . Even more precise data are obtained when the two denaturation curves overlap as there is no extrapolation and values of  $\Delta G_D$  for each protein can be made under identical conditions (for example, wild type and Ile  $\rightarrow$  Val96).

actions where the helix composed of residues 6 to 18 docks on to the  $\beta$ -sheet, effectively introducing cavities into the enzyme. The change in stability on mutation is then measured by the change in free energy of the reversible denaturation of the enzyme, determined by UV difference spectroscopy. We have chosen urea-induced denaturation rather than thermally induced denaturation for two reasons. First, it is not a simple matter to apply thermal denaturation for quantitative comparison of different proteins with significantly different values of  $T_m$ . The enthalpy of unfolding of proteins varies with temperature and the van't Hoff plots are not linear<sup>17</sup>. Thermodynamic quantities are consequently not easily extrapolated to different temperatures. Urea denaturation, however, may be performed at a single temperature for a whole series of mutants (for example, 25 °C as in Fig. 1) and analysed as below<sup>18</sup>. Second, urea denaturation is thought to give a more completely unfolded protein than does thermal denaturation<sup>17,18</sup>.

Wild-type and engineered mutants were expressed in *Escherichia coli*. The yield of mutant enzymes decreases as the mutations become more radical, although specific activity towards hydrolysis of RNA drops by no more than 30%. Mutants Ile96  $\rightarrow$  Ala and Phe7  $\rightarrow$  Leu are expressed at levels of only some 10% of wild type. The decrease in yield presumably reflects a greater susceptibility to proteolytic degradation as the folded structure becomes less stable. Urea denaturation confirms that mutation lowers stability. It can be seen from Fig. 1 that the concentration of urea required to unfold 50% of the protein is 4.63 M for wild-type barnase, 4.19 M for Ile96  $\rightarrow$  Val, 2.90 M for Ile96  $\rightarrow$  Ala and 2.66 M for Phe7  $\rightarrow$  Leu at pH 6.3.

The free energy of unfolding in the absence of urea ( $\Delta G_D^{H_2O}$ ) is conventionally estimated by extrapolating the free energy of unfolding at each individual concentration of urea ( $\Delta G_D$ ) to zero concentration assuming that they are linearly related (equation (1), ref. 18). This procedure gives  $10.5 \pm 0.2$  kcal mol $^{-1}$

$$\Delta G_D = \Delta G_D^{H_2O} - m[\text{urea}], \quad (1)$$

for  $\Delta G_D^{H_2O}$  for wild-type barnase,  $9.3 \pm 0.2$  kcal mol $^{-1}$  for Ile96  $\rightarrow$  Val,  $6.5 \pm 0.1$  kcal mol $^{-1}$  for Ile96  $\rightarrow$  Ala and  $6.44 \pm 0.04$  kcal mol $^{-1}$  for Phe7  $\rightarrow$  Leu. (About 2 parts in  $10^5$  of the latter two mutants are unfolded in the absence of urea.) The slopes of the plots are quite similar for wild type and mutants

( $m = 2.27 \pm 0.04$ ;  $2.22 \pm 0.05$ ,  $2.24 \pm 0.04$  and  $2.42 \pm 0.01$ , respectively). Wild-type barnase is significantly more stable than T1 ribonuclease ( $\Delta G_D^{H_2O} = 4$  kcal mol $^{-1}$ , ref. 18) and staphylococcal nuclease ( $\Delta G_D^{H_2O} = 5.5$  kcal mol $^{-1}$ , ref. 8), so allowing more radically destabilizing mutations to be made and analysed.

While the above procedure is adequate for estimating the approximate value of  $\Delta G_D^{H_2O}$ , the extrapolations are over too wide a range of concentrations of urea to measure reliably small differences in stability of mutants (errors in the slope are magnified and the assumption about precise linearity between free energy of unfolding and [urea] is questionable, although the slope  $m$  is reasonably constant between 2.5 and 5 M urea). We have used two alternative procedures for measuring differences more directly. The first is the better of the two and makes no assumptions about the dependence of unfolding energy on urea concentration. Wild-type and mutant enzymes of suitably similar stability are denatured in parallel at the same concentrations of urea. Then, at each concentration of urea, the difference in  $\Delta G_D$  between wild-type and mutant ( $= \Delta\Delta G_D = \Delta G_{D(wt)} - \Delta G_{D(mut)}$ ) is given directly by:

$$\Delta\Delta G_D = RT \ln \frac{\frac{[\text{folded}]}{[\text{unfolded}]]_{wt}}{[\text{folded}]} \quad (2)$$

This procedure clearly works well for small differences in stability where denaturation curves overlap and the ratio of [folded]/[unfolded] for wild-type and for mutant can be measured under the same conditions. This cannot be applied to situations where there are large differences in stability between two mutants because the ratio of [folded]/[unfolded] can be measured accurately only in the range 0.1 to 10. Consequently, the ratio will be too extreme for an unstable mutant when the ratio is measurable for wild-type. Eventually, the procedure will be scaled over a series of mutants, each one differing from the next in stability by less than a kcal mol $^{-1}$ . Until then, the second procedure may be applied: a small extrapolation is made to a concentration of urea intermediate between the values required for denaturation of wild type and mutant (or between two mutants, c.f. ref. 19, Fig. 2). This assumes linearity in the dependence of  $\Delta G_D$  on [urea] only over a small range beyond the experimental data. Values of  $\Delta\Delta G_D$  are listed in Table 1. Truncating Ile to Val destabilizes wild type by 1.1 kcal mol $^{-1}$ , and Ile to Ala by 4.0 kcal mol $^{-1}$ . Mutation of Phe to Leu destabilizes by 4.6 kcal mol $^{-1}$ . The experiments thus give direct measure-

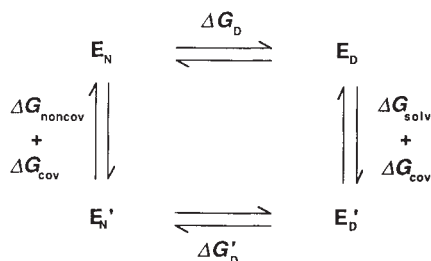
**Table 1**

| Mutant                  | $\Delta\Delta G_D^*$<br>(in urea) | $\Delta\Delta G_D^{H_2O} \dagger$<br>(at 0 M urea)<br>(kcal mol $^{-1}$ ) | $\Delta G_{solv} \ddagger$ |
|-------------------------|-----------------------------------|---------------------------------------------------------------------------|----------------------------|
| Ile $\rightarrow$ Val96 | 1.1                               | 1.2                                                                       | -0.12                      |
| Ile $\rightarrow$ Ala96 | 4.0                               | 4.0                                                                       | -0.15                      |
| Phe $\rightarrow$ Leu7  | 4.6                               | 4.1                                                                       | 2.24                       |

\*  $\Delta G_{D(wt)} - \Delta G_{D(mut)}$  calculated by direct comparison at the same concentrations of urea (wild type and Ile  $\rightarrow$  Val96) or interpolation to an intermediate concentration of urea (Ile  $\rightarrow$  Val96 and Ile  $\rightarrow$  Ala96 at 3.5 M urea, Ile  $\rightarrow$  Ala96 and Phe  $\rightarrow$  Leu7 at 2.8 M urea, see Fig. 2 and text). The similarities in the slopes of the denaturation curves renders the interpolation insensitive to the choice of [urea] within  $\pm 1$  M.

$\dagger \Delta G_D^{H_2O}(wt) - \Delta G_D^{H_2O}(mut)$  from extrapolation to [urea] = 0 M (equation (1)). Note that there is expected to be a small discrepancy between this and the previous column because of the different stabilities of the exposed side chains in the denatured state when exposed to water and urea. For complete exposure of the side chain, this is negligible for Ile  $\rightarrow$  Val, +0.15 kcal mol $^{-1}$  for Ile  $\rightarrow$  Ala and is +0.18 kcal mol $^{-1}$  for Phe  $\rightarrow$  Leu at 4 M urea (the larger side chains being more stable in urea)<sup>25</sup>.

$\ddagger$  Data from ref. 23 for the change in free energy of solvation of side chain ( $G_{solv(mut)} - G_{solv(wt)}$ ).



**Fig. 3** Thermodynamic cycle relating the unfolding of wild-type (E) and mutant (E') enzymes. On mutation, there is a change in the free energy of the protein ( $\Delta G_{\text{cov}}$ ) because of covalent changes such as the change in chemical bonds on going from Ile to Ala).  $\Delta G_{\text{cov}}$  is the same for both the folded and unfolded states and so cancels out when comparing the right and left hand sides of the cycle. There are also changes in the noncovalent interactions,  $\Delta G_{\text{noncov}}$  ( $=G_{E'_N} - G_{E_N}$ ), on mutation. In the unfolded enzyme, the side chains of the amino acids are more, if not completely exposed to solvent and so  $\Delta G_{\text{noncov(D)}}$  represents predominantly the changes in the solvation energy of the side chain,  $\Delta G_{\text{solv}}$  ( $=G_{E'_D} - G_{E_D}$ ), on mutation.  $\Delta G_{\text{noncov}}$  for the folded state represents predominantly the loss of interaction energy between the enzyme and those parts of the side chain that are deleted on mutation.  $\Delta G_{\text{noncov}}$  contains, in addition, the energy terms associated with any reorganization of enzyme and associated solvent on mutation. The experimentally determined quantity in our experiments is the difference in unfolding energy of the wild-type and mutant enzymes,  $\Delta G_D - \Delta G'_D$ . It is seen from the cycle that  $\Delta G_D - \Delta G'_D = \Delta G_{\text{noncov}} - \Delta G_{\text{solv}}$ .

ments of the destabilizing effects of cavities in enzymes. This points to a way of stabilizing protein structures: make substitutions to fill in holes that occur in the native structure.

Calculation of the free energy of folding (or unfolding) of proteins *ab initio* involves determining the free energies of the folded and unfolded states (Fig. 3,  $\Delta G_D = G_{E_D} - G_{E_N}$ , where  $E_N$  and  $E_D$  are the native and denatured forms of the enzyme). This requires calculating all the noncovalent interaction energies in the folded and unfolded proteins and the solvation energies. One fundamental unknown quantity is the noncovalent interaction energy in the unfolded state. This is probably not a single state but consists of a mixture of many different states of similar energies, in which the exposure of side chains to solvent is unknown and variable. This presents a stumbling block to calculation. It is important to relate the empirical data from the protein engineering experiments,  $\Delta \Delta G_D$ , ( $=\Delta G_D - \Delta G'_D$ ) to the energies used in calculations. This is done by a simple thermodynamic cycle (Fig. 3) which considers the native and denatured states for wild-type ( $E_N$  and  $E_D$ ) and mutant ( $E'_N$  and  $E'_D$ ) enzymes. In Fig. 3, the change in the noncovalent interaction energy in the native enzyme on removal of a side chain is defined by  $\Delta G_{\text{noncov}} = G_{\text{noncov(mut)}} - G_{\text{noncov(wt)}}$  (where the subscripts refer to the noncovalent interactions in the folded mutant and wild-type enzymes). The equivalent change in noncovalent energy in the denatured state is termed  $\Delta G_{\text{solv}}$  from Fig. 3:

$$\Delta G_D^{\text{H}_2\text{O}} - \Delta G'_D{}^{\text{H}_2\text{O}} = \Delta G_{\text{noncov}} - \Delta G_{\text{solv}} \quad (3)$$

First, it can be seen from equation (3) that the changes in free energy of unfolding are a function of the interactions in folded and denatured states. Second, equation (3) may be used to gather information about interactions in the denatured state as follows.  $\Delta G_{\text{noncov}}$  may be calculated from the native structures of the wild-type and mutant enzymes using conventional computational methods. Recent developments in computational methods allow the direct calculation of the values of  $\Delta G_{\text{noncov}}$  (and  $\Delta G_{\text{solv}}$ ) by the mathematical equivalent of mutating the side chains in slow increments<sup>20-22</sup>. If the side chain in the unfolded state is completely exposed to solvent, then  $\Delta G_{\text{noncov}}$  is simply calculated from equation (3) using relative solvation energies of the amino acid side chains that are available from

experiments on model compounds<sup>23,24</sup>. For example,  $\Delta G_{\text{solv}}$  for Ile  $\rightarrow$  Val is  $-0.12$ , Ile  $\rightarrow$  Ala is  $-0.15$  and Phe  $\rightarrow$  Leu is  $2.24$  kcal mol<sup>-1</sup> (ref. 23). Thus, if the side chain of Phe7 in denatured wild-type enzyme is completely exposed to solvent and so also is Leu7 in the mutant, then  $\Delta G_{\text{noncov}}$  for Phe  $\rightarrow$  Leu would be  $4.6 + 2.2 = 6.8$  kcal mol<sup>-1</sup>. Discrepancies between calculated and measured values of  $\Delta G_{\text{noncov}}$  will give clues about side chain interactions in the unfolded states. Further mutational experiments will provide additional direct data on protein stability and more general information on interactions in native and denatured states.

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## Errata

### A hypothetical model of the foreign antigen binding site of Class II histocompatibility molecules

J. H. Brown *et al.*

*Nature* **332**, 845-850 (1988).

In Fig. 3a, the I-A<sup>d</sup> sequence numbers 10, 12, 14 should be replaced by the numbers 11, 13, 15 respectively; the word "first" in the seventh line, fourth paragraph of page 846 should be deleted.

### Palaeoenvironmental trends in the history of trace fossils

D. J. Bottjer, M. L. Droser & D. Jablonski

*Nature* **333**, 252-255 (1988).

On page 253, left column, fourth paragraph down, a line of text is missing between the seventh and eighth lines. This line reads "features, and, most important to our study, avoid the circular".

### A cellular analogue of visual cortical plasticity

Y. Frégnac, D. Shulz, S. Thorpe & E. Bienenstock

*Nature* **333**, 367-370 (1988).

The bottom line shown in Fig. 2 should be read in the reverse order, "LEFT RIGHT", instead of "RIGHT LEFT", such as that peri-stimulus histograms presented in the left column correspond to stimulation through the left eye ( $S^+$ ), and those presented in the right column correspond to stimulation through the right eye ( $S^0$ ).